REVIEW ARTICLE

Regulation of photosynthetic gene expression in purple bacteria

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Keywords: photosynthesis, purple bacteria, gene expression, oxygen-sensing, light-sensing

Overview

Purple phototrophic bacteria have the ability to capture and use sunlight efficiently as an energy source. In these organisms, photosynthesis is carried out under anaerobic conditions. The introduction of oxygen into a culture growing phototrophically results in a rapid decrease in the synthesis of components of the photosynthetic apparatus and a change to an alternative source of energy, usually derived from the degradation of organic compounds under aerobic conditions (chemoheterotrophy). Switching back and forth between anaerobic (photosynthetic) and aerobic growth requires tight regulation of photosynthetic gene expression at the molecular level. Initial experiments by Cohen-Bazire *et al.* (1957) showed quite clearly that the regulation of photosynthetic gene expression was in response to two environmental stimuli. The most potent stimulus was oxygen; its presence shut down production of photosynthetic pigments very rapidly. To a lesser extent photosynthetic gene expression responded to light intensity. Low light intensity produced high levels of photosynthetic pigments; high light intensities caused a decrease, but the effect was less dramatic than that observed for oxygen. Since these initial observations were made in *Rhodobacter sphaeroides* some forty years ago, a great deal has been revealed as to the nature of the genes that encode the various components of the photosynthetic apparatus. Recent progress in the understanding of the regulation of expression of these genes in *R. sphaeroides* and *Rhodobacter capsulatus* is the subject of this review.

The photosynthetic apparatus in *R. sphaeroides* and *R. capsulatus*

The photosynthetic apparatus of *Rhodobacter* is relatively simple, consisting of two types of light-harvesting (LH) antennae, LH I and LH II, a reaction centre (RC) complex and various electron transport proteins. LH complexes absorb light in the visible (450–590 nm) and

near infrared (800–875 nm) wavelengths and funnel this excitation energy into RCs where it is converted to electrochemical energy which can be used by the cell. LH I and LH II each consist of two membrane-spanning polypeptides with non-covalently bound bacteriochlorophyll and carotenoid molecules (Kiley & Kaplan, 1988). When growing photosynthetically, *Rhodobacter* exhibits a proliferation of invaginations in the cytoplasmic membrane providing a substantial increase in the overall area of the cellular membranes. The LH complexes and RCs are embedded in these membranes in the form of a series of ring structures. Each RC is surrounded by a ring of 16 LH I molecules, which interact with LH II complexes consisting of rings of nine LH II molecules (Fig. 1).

The photosynthetic gene cluster

Whilst *R. capsulatus*, like most other bacteria, possesses a single main chromosome (4.4 Mb), its close relative *R. sphaeroides* was the first, and is one of only a small

Fig. 1. The effect of light intensity and oxygen tension on the development of the photosynthetic apparatus of *R. sphaeroides*.

Fig. 2. Regulation of the photosynthetic gene cluster of *Rhodobacter* by the aerobic repressor PpsR under aerobic (a) and anaerobic, light (b) conditions. The arrows represent the direction of transcription; the size of the arrows indicate the strength of transcription. (c) Organization of the photosynthetic gene cluster.

number of bacteria known to possess two main chromosomes (3 Mb and 1 Mb) (Suwanto & Kaplan, 1989). Genes encoding the synthesis of bacteriochlorophyll and carotenoid photopigments, RC complexes, and LH I and LH II polypeptides are tightly clustered in a single 80 kb (photosynthetic gene cluster) region of the large main chromosome of both *R. sphaeroides* and *R. capsulatus*, along with many ORFs whose exact functions are yet to be determined (Yen & Marrs, 1976; Pemberton & Bowen, 1981; Taylor *et al.*, 1983; Willison *et al.*, 1985; Armstrong *et al.*, 1989; Coomber & Hunter, 1989; Bollivar *et al.*, 1994; Fig. 2).

Detailed molecular biological analysis of the photosynthetic gene cluster has identified individual genes involved in bacteriochlorophyll synthesis (*bch*), the synthesis of coloured carotenoids (*crt*), RC (*pufLM* and *pufA*), and LH I (*pufBA*) and LH II (*pucBA*) polypeptides. Whilst most of the genes so far analysed occur in a continuous 45 kb segment of the photosynthetic gene cluster, the *puc* operon is located 20 kb outside this segment.

Virtually all of the *crt* genes with the exception of ORF160 (alternatively known as *crtK* or *tspO*) have been cloned, sequenced and allocated a specific function. Although the exact function of ORF160 is unknown, it does have significant sequence identity to the rat benzodiazepine receptor, and may play a role in the negative regulation of either the biosynthesis or assembly of photosystem components in response to oxygen (Yeliseev & Kaplan, 1995). Within the *puf*, *puf* and *puc* operons, there is some debate over the function of PufQ, whilst the exact functions of PufX, PucC, PucD and PucE remain unclear. The complexity of the pathway for the synthesis of bacteriochlorophyll *a* has meant that not all the genes have been identified and allocated functions; genetic analysis of this pathway is more detailed in *R. capsulatus* than in *R. sphaeroides* (Coomber *et al.*, 1990; Bolivar *et al.*, 1994; Lang *et al.*, 1995).

Pemberton & Bowen (1981) were able to demonstrate high-frequency conjugal transfer of the photosynthetic gene cluster mediated by the broad-host-range IncP plasmid RP4 carrying the mercury resistance transposon Tn501. The presence of similar photosynthetic clusters

in *R. sphaeroides*, *R. capsulatus* and other photosynthetic bacteria suggests a role for transposons and broad-host-range plasmids in the recruitment and arrangement of diverse genes to form such a cluster and the subsequent dispersal of this cluster to other bacteria. It may be that there is strong evolutionary pressure for genes involved primarily in the regulation of photosynthesis itself to occur within the photosynthetic cluster, whilst global regulatory genes which integrate photosynthetic activity with other cellular functions may occur in other areas of the main chromosome(s).

It is clear that considerable advances have been made in our understanding of the genes which encode the various elements of the *Rhodobacter* photosynthetic apparatus. However, until relatively recently little was known of the regulators which respond to oxygen and light intensity; this situation has changed dramatically over the last five years.

The oxygen-responsive repressor PpsR

In 1991, Penfold and Pemberton isolated and identified PpsR, an oxygen-responsive transcriptional regulator, the first such regulator of bacterial photosynthesis (Penfold & Pemberton, 1991, 1994). In contrast to some other oxygen-responsive regulators which are activators, PpsR acts as a repressor under aerobic conditions to shut down photosynthetic gene expression.

Under aerobic conditions, *R. sphaeroides* colonies have vibrant red centres with white edges. The centres of the colonies are anaerobic and synthesis of the carotenoid sphaeroidenone is derepressed; the edges of the colony are aerobic, and the oxygen suppresses pigment production. When *ppsR* was first cloned and reintroduced into wild-type *R. sphaeroides* on a broad-host-range plasmid, the phenotypic effect was dramatic; the colonies were no longer vibrant red but became white to buff-coloured due to complete repression of carotenoid synthesis (Penfold & Pemberton, 1991, 1994). This very marked reduction in photopigment synthesis is probably due, in part, to increased PpsR synthesis from multiple copies of *ppsR*.

Mutants deficient in the production of coloured carotenoids are easily isolated and under aerobic conditions

the colonies are slate-grey; such carotenoidless (Crt^-) mutants are termed blue-green because they produce very small amounts of bacteriochlorophyll *a*, whose synthesis is strongly suppressed in the presence of oxygen. Directed mutagenesis of *ppsR* produced dark-red strains which showed a 5- to 10-fold overproduction of both carotenoids and bacteriochlorophylls under aerobic conditions. Whilst PpsR^- mutants of the wild-type (Crt^+) strain showed some instability, PpsR^- mutants of a carotenoidless (Crt^-) mutant were extremely unstable. In $\text{Crt}^+ \text{PpsR}^-$ strains, the overproduction of carotenoids presumably had a strongly photoprotective activity against photo-oxidative killing resulting from the interaction between oxygen and bacteriochlorophyll; in $\text{Crt}^- \text{PpsR}^-$ the absence of carotenoids saw maximal photo-oxidative damage. So lethal was photo-oxidative killing of $\text{Crt}^- \text{PpsR}^-$ strains that attempted serial subculture resulted in a preponderance of mutants blocked at various stages of bacteriochlorophyll synthesis; each of the mutants still overproduced bacteriochlorophyll intermediates preceding the block (Penfold & Pemberton, 1994). Genetically unstable dark-red mutants of *R. sphaeroides* were first isolated almost four decades ago (Griffiths & Stanier, 1956). Three dark-red mutants, isolated via three separate routes, have subsequently been described (Lascelles & Wertlieb, 1971). Whilst these mutants showed normal anaerobic photopigment levels and light regulation, they produced 5- to 50-fold more bacteriochlorophyll and carotenoid under aerobic conditions. More recently, genetically unstable dark-red mutants were isolated that produced increased levels of bacteriochlorophyll, carotenoid and LH II under aerobic conditions (Lee & Kaplan, 1992). Some or all of these mutants are probably deficient in *PpsR*. Sequencing of *ppsR*, which lies between *bchF* and *bchE*, revealed an ORF of 1407 bp encoding a protein of 51 kDa. An examination of the predicted amino acid sequence revealed that the C-terminus possessed an helix–turn–helix (HTH) motif characteristic of DNA-binding proteins. The HTH of *PpsR* shows significant sequence identity with members of the NtrC class of response regulators, and the HTH domains of a variety of regulatory proteins including λCII , λCro , Crp, LacI, GalR, TnpR, AraC and LexA.

Putative *PpsR*-binding sites with the consensus (TGT- N_{12} -ACA) occur upstream of a number of *R. sphaeroides* and *R. capsulatus* photosynthetic genes. This regulatory palindrome shows sequence similarity to the NtrC and NifA consensus sequences (Gussin *et al.*, 1986), a possible binding site for the *Pseudomonas aeruginosa* PilR protein (Hobbs *et al.*, 1993; Pasloske *et al.*, 1989), a possible binding site for the *Caulobacter crescentus* FlbD protein (Ramakrishnan & Newton, 1990), and a prokaryotic consensus sequence (Gicquel-Sanzey & Cossart, 1982), derived from the recognition site of LysR and a number of closely related regulatory proteins.

The prediction that *PpsR* (and its *R. capsulatus* homologue ORF469) is a repressor which binds to these palindromes under aerobic conditions has been verified

experimentally in *R. sphaeroides* where it was shown to bind to the predicted palindromes upstream of *puc*, *bchF*, *bchC* and *crtD* (Penfold & Pemberton, 1994; Gomelsky & Kaplan, 1995a) and in *R. capsulatus* upstream of *bchF*, *bchC*, *crtI*, *CrtE* and *puc* (Ponnampalam *et al.*, 1995). Since the LH II complex is the major photopigment binding site, the co-regulation of *bch*, *crt* and *puc* operons may help to protect the cell from photo-oxidative killing by ensuring a minimal free bacteriochlorophyll pool.

The ability to shut down the synthesis of the photosynthetic apparatus rapidly and completely in the presence of oxygen is greatly facilitated by a regulator that acts simultaneously on many photosystem genes. It is clear that the repressor activity of *PpsR* is switched on by oxygen. What has yet to be determined is how this activation occurs. Does it occur by direct binding of oxygen, or by *PpsR* directly sensing changes in redox potential resulting from changes in oxygen levels? A more complex situation can be envisaged where a sensor(s) detects either changes in oxygen or redox levels and relays these changes to *PpsR*. The answer may lie in one or a combination of these mechanisms. One possible clue comes from the sequence identity between *PpsR* and the direct oxygen sensor FixL of *Sinorhizobium* (previously *Rhizobium*) *meliloti*. FixL is an oxygen-binding haemoprotein and its sequence identity to *PpsR* is in the oxygen-binding N-terminal domain (FixL residues 126–260, *PpsR* residues 105–240; Monson *et al.*, 1992). However, the histidine residue which ligates the Fe in the haem centre of FixL is not present in *PpsR*. The fact that *PpsR* repression is functional in heterologous hosts such as *Paracoccus denitrificans*, *Pseudomonas stutzeri* and *Escherichia coli* suggests that *PpsR* may well respond to O_2 levels directly (Penfold & Pemberton, 1994; Gomelsky & Kaplan, 1995a; J. M. Pemberton & R. J. Penfold, unpublished data).

A recent study by Gomelsky & Kaplan (1997) has shown that AppA, a protein that plays a critical role in the regulation of photosynthesis but has neither an identifiable DNA-binding domain nor any sequence identity with known regulators of gene expression, interacts with *PpsR* to alter its repressor activity. *appA* null mutants show low levels of photosynthetic gene expression and are white under aerobic conditions. *ppsR* null mutants show considerably increased levels of photosynthetic gene expression compared with the wild-type and are deep-red due to overproduction of both carotenoids and bacteriochlorophylls under aerobic conditions. A *ppsR appA* double null mutant behaves like a *ppsR* null mutant, suggesting that AppA interacts with *PpsR* to reduce its activity under aerobic conditions. Characterization of spontaneous suppressor mutations of an *appA* null mutant lend credence to this hypothesis. Most suppressor mutations are located in *ppsR* and result in a range of amino acid substitutions. These mutations fall into three broad classes: those which are postulated to disrupt secondary structure, those which decrease DNA binding and those which might show an altered response to redox changes. It is

also worthy of note that PpsR is an effective light-dependent repressor under photosynthetic (anaerobic) conditions (Gomelsky & Kaplan, 1997) whose activity is still dependent upon AppA. Under these circumstances oxygen is not involved and a 'redox'-generated signal to PpsR through AppA is a likely pathway.

Contiguous with *ppsR* and upstream from it is *ppsS*, a putative regulator of PpsR activity (Penfold & Pemberton, 1994). Sequencing of *ppsS* has shown that it has high amino acid identity with the central region of AppA, indicating that PpsS may interact with, and dampen the effect of, PpsR (see later). PpsS also has amino acid sequence similarity with the receiver domain of ArcB, the aerobic respiration control sensor, suggesting that PpsS may play some role in redox regulation via phosphoryl transfer. Interestingly, the region common to both PpsS and ArcB also occurs in Cgrc-1, the chloroplast-greening protein of *Chlamydomonas reinhardtii*, indicating a possible role for Cgrc-1 in the regulation of *Chlamydomonas* chlorophyll synthesis.

A number of so-called aerobic photosynthetic bacteria that produce bacteriochlorophyll in the presence of oxygen have been isolated (Fuerst *et al.*, 1993). Since many are closely related to photosynthetic species, it is possible that they are derived from phototrophs. Inactivation or deletion of *ppsR* could be a requisite step in such an evolutionary transition. Interestingly, a mutant of *Rhodocista centenaria* (previously *Rhodospirillum centenum*) that, unlike the wild-type, shows oxygen repression of photopigment synthesis, has been isolated (Yildiz *et al.*, 1991, 1992). One possible explanation is that *R. centenaria* possesses a silent copy of *ppsR* which can be activated by mutation.

Super operons

A number of the operons of the photosynthetic gene cluster of *R. capsulatus* have been shown to be arranged in 'super operons' such that adjacent operons are transcribed as a single unit (Bauer *et al.*, 1991; Wellington *et al.*, 1992). For example the *crtEF*, *bchCXYZ* and *puf* operons are transcribed in the same direction and are cotranscribable (Fig. 2). Transcription may begin at the initiation sites of any of these three operons and continue through the downstream operons. Since PpsR can regulate gene activity by binding upstream of either *crtE* and/or *bchC*, it has the ability to indirectly control the downstream *puf* operon which does not appear to have a PpsR-binding motif. Indeed, a plasmid containing the *puf* promoter fused to the *lacZ* gene demonstrated that it is not regulated directly by PpsR (Gomelsky & Kaplan, 1995a). Genes stretching from *bchF* to beyond *puhA* form a similar 'super operon'. Equally, a superoperonal transcript emanating from *bchF* and terminating beyond *puhA* can be controlled by the PpsR-binding motif of *bchF*. Hence, part of the transcription of the *puf* and *puh* operons can be controlled by PpsR, although neither has a PpsR-binding site controlling their individual operon. How-

ever, the major regulator of the *puf* and *puh* operons is the activator Reg/Prr system.

The Reg/Prr sensor histidine kinase response regulator

Sganga & Bauer (1992) made use of the gratuitous induction of the photosynthetic apparatus which occurs during anaerobic dark growth of *R. capsulatus* and *R. sphaeroides* with DMSO as electron acceptor to screen for mutants with decreased levels of pigment under dark anoxic conditions. Analysis of photosynthetic gene expression in Reg mutants under microaerophilic conditions identified a 30-fold lower expression of the *puf*, *puc* and *puh* operons and a 3-fold lower expression of *bchC* and *bchH* genes (as measured by translational fusions of these operons to *lacZ*) in the mutants compared to the level in wild-type cells. The consequence of this decrease in expression of photosynthetic genes was that Reg mutants were unable to grow at very low light intensities, grew at reduced rates, compared to wild-type cells, at medium light intensities and grew at rates which were close to those of wild-type *R. capsulatus* at high light intensities (Sganga & Bauer, 1992). Complementation of Reg mutants identified the gene *regA* as a *trans*-acting regulator of photosynthetic gene expression (Sganga & Bauer 1992). Sequence analysis revealed that RegA had a receiver domain typical of response regulators but, unlike the majority of response regulators, it appeared to lack a conventional DNA-binding domain. The homologue of *regA* in *R. sphaeroides* has also been cloned and sequenced (Phillips-Jones & Hunter, 1994; Eraso & Kaplan, 1994). Eraso & Kaplan (1994) named this gene *prrA* (*prr* = photosynthesis response regulator). Unlike RegA mutants of *R. capsulatus*, PrrA mutants of *R. sphaeroides* were found to be unable to grow under phototrophic conditions at any light intensity. Northern blot analysis by Eraso & Kaplan (1994) confirmed that PrrA positively regulated *puf*, *puc* and *cycA* (the cytochrome *c*₂ structural gene) expression in response to a decrease in oxygen tension.

A sensor histidine kinase which communicates with RegA/PrrA was identified and named RegB in *R. capsulatus* (Mosley *et al.*, 1994) and PrrB in *R. sphaeroides* (Eraso & Kaplan, 1995). These proteins exhibit 58% sequence identity (Eraso & Kaplan, 1995), have six putative membrane-spanning helices, and are typical of proteins of this class (Parkinson, 1993). Mutations in the *regB/prrB* genes produced cells that would only grow phototrophically at high light intensity (Mosley *et al.*, 1994; Eraso & Kaplan, 1995). Analysis of the suppression of the PrrB⁻ phenotype using an *R. sphaeroides* cosmid library has revealed some interesting molecular physiology. Gomelsky & Kaplan (1995b) identified nine cosmids from an *R. sphaeroides* library which partially suppressed the effect of a mutation in *prrB* and thus restored photosynthetic gene expression to varying degrees. One of these cosmids was shown to contain a gene which encoded the HupT sensor histidine kinase, a

sensor which is normally considered to have a role in the regulation of hydrogen uptake (Elsen *et al.*, 1993). These results provide strong evidence for cross-talk between PrrA and a number of sensor histidine kinases, and also explain why *prrA* in multi-copy can partially suppress the effect of the *prrB* mutation on photosynthetic gene expression (Eraso & Kaplan, 1995). Recently, Qian & Tabita (1996) described a conditional mutant of *R. sphaeroides* that required DMSO for phototrophic growth. This turned out to be a RegB/PrrB mutant; the most likely explanation for this phenotype is cross-talk between the sensor kinase for DMSO (DorS) and RegA (I. M. Horne & A. G. McEwan, unpublished observations).

SenC/PrrC, a redox regulator of photosynthetic gene expression

The *reg/prr* genes are transcribed in opposite directions (Fig. 3) and are separated by a third gene known as *senC* in *R. capsulatus* (Buggy & Bauer, 1995) and *prrC* in *R. sphaeroides* (Eraso & Kaplan, 1995). *prrC* is transcribed in the same direction as *prrA* although the details of the transcriptional regulation of the two genes is unknown. The phenotype of a *prrC* mutant is interesting; it retains the ability to grow phototrophically but synthesizes only about 20% LH II and 50% LH I when grown anaerobically on DMSO in the dark (Eraso & Kaplan, 1995). PrrC appears to be part of the PrrB/PrrA signal transduction pathway or it converges on this pathway; whenever *prrB* or *prrA* was placed *in trans* in a *prrC* null mutant the effect of the mutation was always seen and there appears to be a synergism in the effect of mutations of *prrB* and *prrC* on LH complexes (Eraso & Kaplan, 1995).

The structure of PrrC is also interesting; Eraso & Kaplan (1995) have reported that although PrrC is membrane-bound, analysis of PhoA fusions indicates that the C-terminus is in the periplasm. Sequence analysis has revealed that PrrC has a high degree of sequence identity to two genes from *Saccharomyces cerevisiae*, *sco1* and *sco2* which are essential for the accumulation of cytochrome oxidase in the mitochondrion (Schulze & Rodell, 1989). SenC has 33% sequence identity to *Sco1* (Buggy & Bauer, 1995) and further sequence analysis revealed that it possesses a sequence motif characteristic of bacterial ferredoxins (Buggy & Bauer, 1995). This certainly suggests that its activity might be influenced by electron transfer reactions, perhaps by components of the photosynthetic and respiratory chain. *prrC* homologues have also been identified in the Mn²⁺-oxidizing gene cluster of a marine *Bacillus* and in two rickettsias (van Waasbergen *et al.*, 1996), indicating that this gene may be widespread in bacterial genera.

PrrA is a global regulator of gene expression

Although it is clear that PrrA is essential for high levels of photosynthetic gene expression in *Rhodobacter* its mechanism of action remains elusive. One possibility is

that PrrA itself is a DNA-binding protein, but in view of its structure it is more likely that PrrA is a component of a complex phospho-relay system in the cell. PrrA is the receiver of environmental signals from a variety of sensor histidine kinases, in addition to PrrB (Gomelsky & Kaplan, 1995b), and it now appears that this response regulator has a truly global role in the regulation of anaerobic gene expression in *R. sphaeroides*. Tabita and co-workers have shown that mutations in *prrB* blocked transcription of the *cbb* regulon which contains RubisCo genes and other genes of the Calvin-Benson-Bassham (CBB) cycle of CO₂ fixation (Qian & Tabita, 1996). Recently, it has been shown that under some circumstances *nif* expression is also positively regulated by the Prr system (Joshi & Tabita, 1996). Thus, the Prr system may integrate the expression of genes involved in phototrophy, diazotrophy and autotrophy (Fig. 4). The response regulators which form the final step in the activation of gene expression associated with these three processes have not been identified. However, in the case of photosynthetic gene expression it is possible that another response regulator, PetR, might be a potential phosphoryl receiver from RegA/PrrA. The *petR* gene is located upstream of the *fbc* operon (encoding the cytochrome *bc*₁ complex) and encodes a typical response regulator with a DNA-binding motif (Tokito & Daldal, 1992). Mutations in *petR* appear to be lethal and so until conditional mutants are generated the precise role of PetR in photosynthetic gene expression cannot be easily assessed. In addition to activating photosynthetic gene expression via phosphorylation of positively acting response regulators, another possibility is that PrrA could also regulate some operons (*puc*, *bch* and *crt* operons) by antagonizing the action of the repressor PpsR. PpsS (known as ORF192 in *R. capsulatus*) and/or AppA might mediate this process; it is established that a mutation in *orf192* caused *R. capsulatus* to produce decreased amounts of photosynthetic pigments (Bollivar *et al.*, 1994). We have made a similar observation for the *ppsS* mutant of *R. sphaeroides* (I. M. Horne, T. A. Williams, A. G. McEwan & J. M. Pemberton, unpublished observations).

What signals does the Prr system sense?

In vitro autophosphorylation of a truncated form of RegB and transfer of the phosphoryl group to purified RegA from *R. capsulatus* confirmed that the Reg/Prr proteins act as a typical two-component system (Inoue *et al.*, 1995). Inoue *et al.* (1995) also reported that ferricyanide inhibited *in vitro* phosphoryl transfer from RegB to RegA and proposed that RegB might be a redox sensor whose histidine kinase activity was abolished following oxidation by ferricyanide. However, ferricyanide is a rather non-specific oxidant and the experiment was conducted using a truncated RegB which lacked all of its membrane-spanning regions. In view of the limitations of this experiment, the question of what RegB (PrrB) senses is still an open one. Eraso & Kaplan (1995) have shown that a point mutation in PrrB (PrrB78 mutation) is sufficient to cause *R. sphaeroides* cells to

Fig. 3. The Reg gene cluster of *R. capsulatus* and Prr cluster of *R. sphaeroides*. The arrows represent the direction of transcription

Fig. 4. Regulation of gene expression by the Prr system in *R. sphaeroides*.

produce high levels of LH complexes even in the presence of oxygen. An attractive explanation which they put forward was that PrrB possessed a phosphatase activity which was active towards PrrA in the presence of oxygen in wild-type cells and prevents a high level of phosphorylated PrrA being formed via histidine kinase activity of PrrB. Loss of PrrB phosphatase activity in the PrrB78 mutation would result in the manifestation of the PrrB kinase activity.

It seems likely that, depending on environmental signals, PrrB is in a kinase-dominant state (anaerobiosis) or a phosphatase-dominant state (aerobiosis) and, although the signals differ, the analogy with the histidine kinase EnvZ springs to mind here (Pratt & Silhavy, 1995). Unlike the situation for EnvZ, the nature of the signals which control PrrB kinase activity are still unclear. Redox-dependent control cannot be ruled out, but neither can the possibility that PrrB senses the magnitude of the proton electrochemical gradient ($\Delta\mu_{H^+}$) across the cytoplasmic membrane or a change in the level of some metabolite in response to anaerobiosis. A similar problem is faced in identifying the environmental signals which are sensed by ArcB in *E. coli* (Iuchi & Linn, 1995).

PrrC may also be important in redox control of photosynthetic gene expression (Eraso & Kaplan, 1995).

Although the presence of an [Fe-S] cluster in PrrC needs to be established such a redox centre would enable PrrC to interact with periplasmic cytochromes. A series of experiments have suggested that photosynthetic gene expression is affected by electron transfer to terminal reductases which lie downstream of the cytochrome *bc*₁ complex and utilize *c*-type cytochromes as electron donors. In our laboratory we have shown that the presence of the oxidant nitrous oxide (N₂O) during phototrophic growth of *R. sphaeroides* causes a reduction in the level of LH complexes (Horne *et al.*, 1996). Furthermore, cells grown in the presence of N₂O show only a small increase in LH complexes when they are grown at lower light intensities. The effect of N₂O is to make the cells behave as though they are always growing at high light intensity, and their failure to adjust their LH levels in response to a decrease in light intensity resembles the phenotype of a RegB mutant in *R. capsulatus* (Sganga & Bauer, 1992). The pathway of N₂O respiration is well-defined (Richardson *et al.*, 1989, 1991) and involves a periplasmic N₂O reductase which requires cytochrome *c*₂ as an electron donor. Electron transfer to N₂O involves both of these cytochromes (Richardson *et al.*, 1991). This has led us to propose that an important signal in the regulation of photosynthetic gene expression is the redox state of a high-potential cytochrome (Horne *et al.*, 1996). We have proposed that N₂O exerts its effect by maintaining a high-potential cytochrome in a relatively oxidized state, at least in relation to its oxidation state in the absence of this electron acceptor, and that the redox state of the cytochrome acts as a signal affecting photosynthetic gene expression.

R. sphaeroides possesses three terminal oxidases: a quinol-oxidizing enzyme, a cytochrome *aa*₃ and a cytochrome *cbb*₃ (Garcia-Horsman *et al.*, 1994). The latter two oxidases are members of the cytochrome oxidase superfamily and take electrons from high-potential *c*-type cytochromes in an electron transport pathway that involves the cytochrome *bc*₁ complex. However, the two cytochrome oxidases are differentially expressed in response to oxygen tension; the cytochrome *aa*₃ is expressed under highly aerobic conditions while under low oxygen tensions cytochrome *cbb*₃ prevails. A pattern of distribution and expression of cytochrome *cbb*₃ in bacteria is emerging which indicates that this oxidase is associated with bacterial growth at low oxygen tensions (Poole, 1994). This is consistent with

the very low K_m that this oxidase has for oxygen. Within the context of photosynthetic gene expression, cytochrome *cbb₃* is interesting because it has recently been found that a mutation in the *cco* operon (encoding cytochrome *cbb₃*) causes increased synthesis of LH complexes under aerobic conditions (Zeilstra-Ryalls & Kaplan, 1996). Buggy & Bauer (1995) have also shown that a similar signal transduction system must operate in *R. capsulatus*; in a mutant which lacked cytochrome *c* oxidase activity (this bacterium does not possess cytochrome *aa₃*) there was an increase in the level of LH complex and the expression of *puf* and *puh* operons in semi-aerobically grown cells. Interestingly, it was also observed by Buggy & Bauer (1995) that photosynthetic gene expression was increased compared to wild-type cells even under anaerobic conditions in a mutant *R. capsulatus* M7, which lacks cytochrome *cbb₃*. Since it is known that a PrrC mutant has much lower pigment levels than the wild-type under phototrophic conditions it is possible that it is PrrC which is responsive towards changes in the redox state of a high-potential cytochrome(s).

Hvr proteins – light-responsive transcriptional regulators

Adjacent to *regA* in the *R. capsulatus* regulatory gene cluster (Fig. 3) are genes which appear to be involved in the light-dependent regulation of photosynthetic gene expression. *hvrA* encodes a small polypeptide (molecular mass 11520 Da) with a pI of 10.2. It is a DNA-binding protein that has been reported to bind to the *puf* and *puh* promoter regions. Mutation of *hvrA* resulted in a failure of *R. capsulatus* to increase *puf* and *puh* expression in response to decreased light intensity (Buggy *et al.*, 1994a); *puc* expression does not appear to be affected by *hvrA*. Thus, an HvrA mutant grows poorly under dim light conditions. Although HvrA affects photosynthetic gene expression, like the Reg/Prr system, it may have a more global role and recently it has been observed that a mutation in *hvrA* also affects *nif* gene expression in *R. capsulatus* (cited in Masepohl & Klipp, 1996). The way in which HvrA activates *puf* and *puh* expression is not clear, although the small size of this histone-like protein and its high pI suggest that it might work by altering DNA topology. The signal which HvrA responds to has also not been identified.

Buggy *et al.* (1994b) have also described another gene, *hvrB*, which encodes a DNA-binding protein of the LysR family (Fig. 3). Mutational analysis has shown that HvrB autoregulates its own expression and activates the expression of the *ahcY* in response to low light and *orf5* of unknown function in response to high light. *ahcY* encodes S-adenosyl-L-homocysteine hydrolase and has been shown to be essential for growth of *R. capsulatus* on minimal medium (Sganga *et al.*, 1992). S-Adenosyl-L-homocysteine (AdoHcy) is the product of transmethylation reactions involving S-adenosyl-L-methionine (AdoMet) and is a potent inhibitor of AdoMet-mediated methyl group transfer reactions. AdoHcy is a non-competitive inhibitor of the enzyme

AdoMet-magnesium protoporphyrin monomethyltransferase and thus the regulation of this methylation reaction, the first committed step in Bch biosynthesis, is of great importance. As a result, it appears that the regulation of the ratio AdoMet/AdoHcy is a potential controlling factor in bacteriochlorophyll synthesis, with a high ratio favouring bacteriochlorophyll biosynthesis. Members of the LysR family usually require a specific metabolite as a co-regulator and this compound has not been identified in relation to HvrB.

In *R. sphaeroides* a rather different approach has resulted in the identification of genes involved in light regulation. Takimiya and co-workers showed that blue light (λ around 450 nm) caused maximal repression of expression of the *puf* and *puc* operons under conditions of low oxygen tension (Shimada *et al.*, 1992) and went on to purify a *trans*-acting factor which bound to a DNA fragment in the *puf* regulatory region (Shimada *et al.*, 1993) which had earlier been shown to function as an oxygen-regulated promoter (Hunter *et al.*, 1991). The gene encoding this *trans*-acting regulator has been identified (Shimada *et al.*, 1996) and has been named *spb* (Fig. 3). Spb appears to be the counterpart of *R. capsulatus* HvrA; the proteins exhibit 58% sequence identity. However, it has been suggested that Spb is a negative effector of *puf* gene expression and that it mediates the blue-light-dependent repression of *puf* and *puc* expression, but as yet no analysis of an Spb mutant has been reported.

Fnr – activator of photosynthetic gene expression in *R. sphaeroides*

Experiments over more than a decade have established the importance of Fnr in the regulation of anaerobic gene expression in *E. coli* and homologues of this protein have now been identified in many facultative anaerobic bacteria (Spiro, 1994). The most common function for Fnr is as an activator of the transcription of genes in response to a decrease in oxygen tension and, in view of this functional role, it was long suspected that an Fnr-like protein might have a role in the activation of photosynthetic gene expression. Although an Fnr homologue with a role in the activation of the expression of genes involved in aromatic hydrocarbon degradation (but not photosynthetic gene expression) in *Rhodospseudomonas palustris* was identified in 1992 (Dispensa *et al.*, 1992), the identification of an *fnr* gene in *R. sphaeroides* is more recent (Zeilstra-Ryalls & Kaplan, 1995). The gene has been designated *fnrL* in honour of Dr June Lascelles, who was pre-eminent in the field of photosynthetic bacterial research for many years.

Inactivation of the *fnrL* gene causes *R. sphaeroides* to exhibit a photosynthetic-negative (PS⁻) phenotype but the mutant is able to grow aerobically, as expected (Zeilstra-Ryalls & Kaplan, 1995). The reason for this phenotype becomes clear when it is recognized that consensus Fnr-binding sites have been identified upstream of *hemA*, one of the isozymes encoding δ -aminolaevulinate (ALA) synthase (Neidle & Kaplan,

1993) as well as *puc* (Lee & Kaplan, 1992). In addition, a consensus FNR-binding sequence was identified upstream of *hemF* (Zeilstra-Ryalls & Kaplan, 1995), a gene encoding one of two coproporphyrinogen-III oxidase isozymes in *R. sphaeroides* (Coomber *et al.*, 1992). The effect of a mutation in *fnrL* would be to prevent an increase in expression of the *hemA* gene which normally occurs upon a decrease in oxygen tension, and since the ALA-synthase-catalysed reaction is the first step in bacteriochlorophyll biosynthesis this would have a dramatic effect on photosynthetic gene expression. In addition, the inability to express *hemF* in an *fnrL* mutant means that a common step of haem and bacteriochlorophyll synthesis would also be blocked. The view that anaerobic expression of *hemF* is essential for bacteriochlorophyll synthesis comes from the observation that a mutation in *hemF* has a PS⁻ phenotype (Coomber *et al.*, 1992). Presumably, under aerobic conditions the alternative coproporphyrinogen-III oxidase isozyme is expressed and this allows haem biosynthesis under these conditions. The control of *hemA* expression by Fnr in *R. sphaeroides* is different from the situation in *R. capsulatus* where no Fnr consensus binding site was reported (Biel *et al.*, 1988; Hornberger *et al.*, 1990). However, it seems almost certain that an *fnr* homologue will be present in *R. capsulatus* and information about its role in photosynthetic gene expression is eagerly awaited.

Fnr and the cyclic AMP receptor protein (CRP) are members of a family of transcriptional regulators which differ in their signalling mechanisms (Spiro, 1994). Thus, the Fnr from *E. coli* is now established to lack a cAMP-binding site, but instead it possesses one [4Fe-4S] cluster per monomer (Lazazzera *et al.*, 1996). The active form of Fnr is a dimer (Ziegelhoffer & Kiley, 1995) and there is now evidence that the extreme oxygen lability of the [4Fe-4S] cluster is associated with monomer formation and decreased ability to bind DNA (Lazazzera *et al.*, 1996). These observations were the development of an elegant series of experiments by Kiley and co-workers which identified and analysed point mutations in the *fnr* gene that allowed Fnr to act as a transcriptional activator under aerobic conditions (Kiley & Reznikoff, 1991; Lazazzera *et al.*, 1996). In summary, it was found that formation of these Fnr* mutants involved two types of point mutation that stabilized the dimeric form of Fnr. This occurred either by increasing the stability of the cysteine-rich [Fe-S] cluster domain towards oxygen or by strengthening monomer-monomer interactions in the so-called allosteric domain (Ziegelhoffer & Kiley, 1995). In one *E. coli* Fnr* protein, LH28-DA154, two point mutations, an Asp to Ala mutation at position 154 and a Leu to His at position 28, led to formation of a more stable [Fe-S] cluster. This allows the dimeric Fnr* to exist in air (Ziegelhoffer & Kiley, 1995). Zeilstra-Ryalls & Kaplan (1995) have pointed out that an Ala residue at position 154 is already present in wild-type *R. sphaeroides* FnrL whilst a His residue is located at position 29. It is tempting to suggest that this may mean that the [Fe-S] cluster of *R. sphaeroides* FnrL may be

more stable towards oxygen than its equivalent in *E. coli* Fnr.

It has been proposed that control of Fnr by oxygen involves the disassembly of the [4Fe-4S] cluster rather than a change in redox state of the Fnr protein (Beinert & Kiley, 1996). The observation that Fnr is oxygen-labile *in vitro* has led to the view that destruction of the [4Fe-4S] clusters by oxygen or oxygen-derived species may also occur *in vivo*. This idea has been strengthened by the observation, using Mossbauer spectroscopy, that oxygen causes the [4Fe-4S] cluster of Fnr to collapse to a [2Fe-2S] cluster (Khoroshilova *et al.*, 1997). This raises questions about the processes which are involved in rebuilding the [4Fe-4S] cluster of Fnr. The reassembly of the [4Fe-4S] cluster in Fnr may be the process where redox control of Fnr is exerted and there is a body of data which suggests the respiratory chain may be important in this process. Even in *E. coli* there is evidence that redox reactions influence Fnr-dependent expression. Unden *et al.* (1990) showed that, under anoxic conditions, positive redox potentials (+440 mV at pH 7) were sufficient to decrease Fnr-dependent expression of an *frd-lacZ* fusion in *E. coli*. Although it might be objected that ferricyanide could directly destroy the [Fe-S] cluster of Fnr, this view is not compelling because cells are relatively impermeable to ferricyanide. However, in *R. sphaeroides* stronger evidence is emerging which suggests that the respiratory chain can influence Fnr.

Zeilstra-Ryalls & Kaplan (1996) showed that *R. sphaeroides cco* mutants which lack the *cb*-type cytochrome oxidase exhibit increased expression of FnrL-dependent genes in the presence of oxygen. *hemA* expression has been shown to be under control of FnrL (Zeilstra-Ryalls & Kaplan, 1995) and its expression under aerobic conditions was increased in the *cco* mutant. In a series of complementary experiments we have shown that the presence of N₂O during phototrophic growth is correlated with a decrease in *hemA* expression under the same conditions (Horne *et al.*, 1996). Like the *cb*-type cytochrome oxidase, N₂O reductase takes electrons from *c*-type cytochromes (in the case of *R. capsulatus* it is cytochrome *c₂*) (Richardson *et al.*, 1991) and this has led us to suggest that redox changes occurring between the cytochrome *bc₁* complex and the terminal reductases affect FnrL activity (Horne *et al.*, 1996). Thus, in *R. sphaeroides* the region of the electron transfer chain between the cytochrome *bc₁* complex and the terminal oxidases could be involved in the regulation of Fnr and the Prr system.

The available data suggests that Fnr in *R. sphaeroides* may be inactivated by oxygen but is also influenced by the electron transfer chain. We suggest that reassembly of the [4Fe-4S] cluster in Fnr is regulated via redox reactions associated with respiratory electron flow. This process probably requires ferrous iron and sulfide, although there is not a great deal of information about [Fe-S] cluster assembly *in vivo*. In the case of iron metabolism it is tempting to suggest a role for the Rdx proteins. Rdx proteins are homologous to NapG in *E.*

Fig. 5. Model for the interaction of Fnr with the respiratory chain via the RdxB protein. (a) Anaerobic conditions; electron flow from cytochrome bc_1 complex to RdxB in the absence of an external electron acceptor. Fnr is maintained in an active state. (b) Aerobic conditions; electron flow from cytoplasmic reductants via RdxB to cytochrome cbb_3 leads to inactivation of Fnr. Dashed arrows indicate that a direct redox interaction between Fnr and the electron transfer chain is not suggested.

coli and proteins of this family are found in a number of facultative aerobes (Berks *et al.*, 1995). The *rdxBHIS* operon, *hemA* operon and *cco* operon in *R. sphaeroides* all have consensus Fnr-binding sequences, and are located in a gene cluster which also contains *fnrL* (Zeilstra-Ryalls & Kaplan, 1995). Examination of the primary structure of Rdx proteins shows that, in addition to two putative [4Fe-4S] clusters there is a cluster of four cysteine residues which could form a rubredoxin-like centre (Neidle & Kaplan, 1992). Recently, O'Gara & Kaplan (1997) have shown that a mutation in *rdxB* resulted in increased photosynthetic gene expression under aerobic conditions. This suggests that RdxB might be involved in connecting Fnr with the respiratory chain, albeit in an indirect way. One possibility is that in *R. sphaeroides* the stability of the [4Fe-4S] cluster in Fnr is regulated by the RdxB and cytochrome cbb_3 oxidase as suggested in Fig. 5 via an effect on the cytoplasmic iron pool. It is tempting to propose that RdxB is involved in the handling of iron and this relates to the assembly of

the [Fe-S] cluster in Fnr. *R. capsulatus* has been shown to reduce a variety of iron(III) chelates and the pathway of electron flow to this ion involves the cytochrome bc_1 complex (Dobbin *et al.*, 1996); it would be interesting to know whether an RdxB mutant of *R. sphaeroides* is able to reduce these iron(III) chelates.

Concluding remarks

The major regulatory components involved in the regulation of photosynthetic gene expression have been identified. They are the oxygen-responsive repressor PpsR and proteins which modulate its activity, such as AppA, the Reg/Prr activating system and at least in *R. sphaeroides*, Fnr. Two lines of research should provide a fascinating insight into the regulation of photosynthetic gene expression; the first is the *in vitro* characterization of the molecular properties of the regulatory systems and here the excellence of photosynthetic bacteria as a system for bioenergetic studies (Jackson, 1988) will be of great importance. The second aim is to understand how the various regulatory systems interact; this is a more difficult task. The classic paper of Cohen-Bazire *et al.* (1957) launched the quest to identify the 'redox' regulators of photosynthetic gene expression. It is interesting, but not surprising in view of the unity of biochemistry and molecular biology, that some of the photosynthetic regulatory systems (for example Fnr) are also used in many non-photosynthetic facultative aerobes. A question which is now raised is whether there are homologues of the PpsR/AppA and Reg/Prr systems in non-photosynthetic bacteria.

Notes added in proof

1. The RegA/PrrA protein has recently been reported to have an HTH at its C-terminus and to bind to DNA sequences upstream of the *puf* and *puc* transcriptional start sites (Kirndorfer *et al.*, 1997). This means that it may not be necessary to postulate that a complex phospho-relay cascade is involved in signal transduction via the Reg/Prr system.

2. It has been reported that *sco1* and *sco2*, the mitochondrial homologues of Prr/SenC, may be Cu-binding proteins which may play a role in the insertion of Cu into cytochrome oxidase (Glerum *et al.*, 1996). The sequence containing the conserved cysteines in these proteins resembles the sequence of the Cu_A -binding region of subunit 2 of mitochondrial cytochrome oxidase. Thus, PrrC/SenC may contain Cu and not an [Fe-S] cluster. Other factors may influence the insertion of Cu into the Cu_B site of cytochrome cbb_3 ; in *Bradyrhizobium japonicum* FixI (the homologue of RdxI in *R. sphaeroides*) has been shown to be required for biogenesis of cytochrome cbb_3 (Preisig *et al.*, 1996) *Arch Microbiol* 165, 297–305]. *fixI* encodes a P-type ATPase and it has been suggested that it is involved in Cu^{2+} export. In view of these data it is possible that two pairs of cysteines of RdxB may also bind Cu^{2+} rather than Fe^{2+} as suggested in this review. Thus, PrrC/SenC and the Rdx systems may influence photosynthetic gene

expression by affecting the biogenesis of cytochrome *cbh₃*. However, the molecular basis for the modulation of the Prr/Reg sensor regulator and of Fnr by these proteins is still unclear.

References

- Armstrong, G. A., Alberti, M., Leach, F. & Hearst, J. E. (1989). Nucleotide sequence, organization and nature of the protein products of the carotenoid biosynthesis gene cluster of *Rhodobacter capsulatus*. *Mol Gen Genet* **216**, 254–268.
- Bauer, C. E., Buggy, J. J., Yang, Z. & Marrs, B. L. (1991). The superoperon organization of genes for pigment biosynthesis and reaction center proteins is a conserved feature in *Rhodobacter capsulatus*: analysis of overlapping *bchB* and *pufA* transcripts. *Mol Gen Genet* **228**, 433–444.
- Beinert, H. & Kiley, P. J. (1996). Redox control of gene expression involving iron–sulfur proteins. Change of oxidation-state or assembly/disassembly of the Fe-S clusters? *FEBS Lett* **382**, 218–219.
- Berks, B. C., Ferguson, S. J., Moir, J. W. B. & Richardson, D. J. (1995). Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions. *Biochim Biophys Acta* **1232**, 97–173.
- Biel, S. W., Wright, M. S. & Biel, A. J. (1988). Cloning of the *Rhodobacter capsulatus hemA* gene. *J Bacteriol* **170**, 4832–4834.
- Bollivar, D. W., Suzuki, J. Y., Beatty, J. T., Dobrowski, J. & Bauer, C. E. (1994). Directed mutagenesis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus*. *J Mol Biol* **237**, 622–640.
- Buggy, J. & Bauer, C. E. (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.
- Buggy, J., Sganga, M. W. & Bauer, C. E. (1994a). Nucleotide sequence and characterization of the *Rhodobacter capsulatus hvrB* gene: HvrB is an activator of S-adenosyl-L-homocysteine hydrolase expression and is a member of the LysR family. *J Bacteriol* **176**, 61–69.
- Buggy, J., Sganga, M. W. & Bauer, C. E. (1994b). Characterization of a light-responding trans-activator responsible for differentially controlling reaction center and light-harvesting I gene expression in *Rhodobacter capsulatus*. *J Bacteriol* **176**, 6936–6943.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulfur bacteria. *J Cell Comp Physiol* **49**, 25–68.
- Coomber, S. A. & Hunter, C. N. (1989). Construction of a physical map of the 45 kb photosynthetic gene cluster of *Rhodobacter sphaeroides*. *Arch Microbiol* **151**, 454–458.
- Coomber, S. A., Chaudhri, M., Connor, A., Britton, G. & Hunter, C. N. (1990). Localized transposon Tn5 mutagenesis of the photosynthetic gene cluster of *Rhodobacter sphaeroides*. *Mol Microbiol* **4**, 977–989.
- Coomber, S. A., Jones, R. M., Jordan, P. M. & Hunter, C. N. (1992). A putative anaerobic coproporphyrinogen III oxidase in *Rhodobacter sphaeroides*. I. Molecular cloning, transposon mutagenesis and sequence analysis of the gene. *Mol Microbiol* **6**, 3159–3169.
- Dispensa, M., Thomas, C., Kim, M.-K., Perrotta, J. A., Gibson, J. B. & Harwood, C. S. (1992). Anaerobic growth of *Rhodospseudomonas palustris* on 4-hydroxybenzoate is dependent of AadR, a member of the cyclic AMP receptor class of transcriptional regulators. *J Bacteriol* **174**, 5803–5813.
- Dobbin, P. S., Warren, L. H., Cook, N. J., McEwan, A. G., Powell, A. K. & Richardson, D. J. (1996). Dissimilatory iron(III) reduction by *Rhodobacter capsulatus*. *Microbiology* **142**, 765–774.
- Elsen, S., Richaud, P., Colbeau, A. & Vignais, P. M. (1993). Sequence analysis and interposon mutagenesis of the *hupT* gene, which encodes a sensor protein involved in repression of hydrogenase synthesis in *Rhodobacter capsulatus*. *J Bacteriol* **175**, 7404–7412.
- Eraso, J. M. & Kaplan, S. (1994). *prpA*, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter sphaeroides*. *J Bacteriol* **176**, 32–43.
- Eraso, J. M. & Kaplan, S. (1995). Oxygen-insensitive synthesis of photosynthetic membranes of *Rhodobacter sphaeroides*: a mutant histidine kinase. *J Bacteriol* **177**, 2695–2706.
- Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.
- Garcia-Horsman, J. A., Barquera, B., Rumbley, J., Ma, J. & Gennis, R. B. (1994). The superfamily of heme–copper respiratory oxidases. *J Bacteriol* **176**, 5587–5600.
- Gicquel-Sanzey, B. & Cossart, P. (1982). Homologies between different procaryotic DNA-binding regulatory proteins and between their sites of action. *EMBO J* **1**, 591–595.
- Glerum, D. M., Shtank, A. & Tzagoloff, A. (1996). *SCO1* and *SCO2* act as high copy number suppressors of a mitochondrial copper recruitment defect in *Saccharomyces cerevisiae*. *J Biol Chem* **271**, 20531–20535.
- Gomelsky, M. & 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. & Kaplan, S. (1995b). Isolation of regulatory mutants in photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1 and partial complementation of a PrrB mutant by the HupT histidine-kinase. *Microbiology* **141**, 1805–1819.
- Gomelsky, M. & Kaplan, S. (1997). Molecular genetic analysis suggesting interactions between AppA and PpsR in regulation of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. *J Bacteriol* **179**, 128–134.
- Griffiths, M. & Stanier, R. Y. (1956). Some mutational changes in the photosynthetic pigment systems of *Rhodospseudomonas sphaeroides*. *J Gen Microbiol* **14**, 698–715.
- Gussin, G. N., Ronson, C. W. & Ausubel, F. M. (1986). Regulation of nitrogen fixation genes. *Annu Rev Genet* **20**, 567–591.
- Hobbs, M., Collie, E. S. R., Free, P. D., Livingston, S. P. & Mattick, J. P. (1993). PilS and PilR, a two-component transcriptional regulatory system controlling expression of type-1 fimbriae in *Pseudomonas aeruginosa*. *Mol Microbiol* **7**, 669–682.
- Hornberger, U., Liebetanz, R., Tichy, H. V. & Drews, G. (1990). Cloning and sequencing of the *hemA* gene of *Rhodobacter capsulatus* and isolation of a delta-aminolevulinic acid-dependent mutant strain. *Mol Gen Genet* **221**, 371–378.
- Horne, I. M., Pemberton, J. M. & McEwan, A. G. (1996). Photosynthesis gene expression in *Rhodobacter sphaeroides* is regulated by redox changes which are linked to electron transport. *Microbiology* **142**, 2831–2838.
- Hunter, C. N., McGlynn, P., Ashby, M. K., Burgess, J. G. & Olsen, J. D. (1991). DNA sequencing and complementation/deletion analysis of the *bchA–puf* operon region of *Rhodobacter sphaeroides*: *in vivo* mapping of the oxygen-regulated *puf* promoter. *Mol Microbiol* **5**, 2649–2661.
- Inoue, K., Kouadio, J. L., Mosley, C. S. & Bauer, C. E. (1995). Isolation and *in vitro* phosphorylation of sensory transduction

components controlling anaerobic induction of light harvesting and reaction center gene expression in *Rhodobacter capsulatus*. *Biochemistry* 34, 391–396.

Iuchi, S. & Linn, E. C. C. (1995). Signal transductions in the Arc system for control of operons encoding aerobic respiratory enzymes. In *Two-component Signal Transduction*, pp. 223–231. Edited by J. H. Hoch & T. J. Silhavy. Washington, DC: American Society for Microbiology.

Jackson, J. B. (1988). Bacterial photosynthesis. In *Bacterial Energy Transduction*, pp. 317–376. Edited by C. Anthony. London: Academic Press.

Joshi, H. M. & Tabita, F. R. (1996). A global, two component signal transduction system that integrates the control of photosynthesis, carbon dioxide fixation and nitrogen fixation. *Proc Natl Acad Sci USA* 93, 14515–14520.

Khoroshilova, N., Popescu, C., Munck, E., Beinert, H. & Kiley, P. J. (1997). Iron-sulfur cluster disassembly in the Fnr protein of *Escherichia coli* by O₂: [4Fe–4S] to [2Fe–2S] conversion with loss of biological activity. *Proc Nat Acad Sci USA* 94, 6087–6092.

Kiley, P. J. & Kaplan, S. (1988). Molecular genetics of photosynthetic membrane biosynthesis in *Rhodobacter sphaeroides*. *Microbiol Rev* 52, 50–69.

Kiley, P. J. & Reznikoff, W. S. (1991). Fnr mutants that activate gene expression in the presence of oxygen. *J Bacteriol* 173, 16–22.

Kirndorfer, M., Hebermehl, M., Hemschemeier, K., Jager, A., Ebel, U. & Klug, G. (1997). Transcriptional regulation of *puf* and *puc* operon expression in *Rhodobacter capsulatus* by the DNA binding proteins RegA and IHF. *Abstracts of the 9th International Symposium on Phototrophic Prokaryotes, Vienna*, p. 69.

Lang, H. P., Cogdell, R. J., Takaichi, S. & Hunter, C. N. (1995). Complete DNA sequence, specific Tn5 insertion map, and gene assignment of the carotenoid biosynthesis pathway of *Rhodobacter sphaeroides*. *J Bacteriol* 177, 2064–2073.

Lascelles, J. & Wertlieb, D. (1971). Mutant strains of *Rhodospirillum rubrum* which form photosynthetic pigments aerobically in the dark; growth characteristics and enzymic activities. *Biochim Biophys Acta* 226, 328–340.

Lazazzera, B. A., Beinert, H., Khoroshilova, N., Kennedy, M. C. & Kiley, P. J. (1996). DNA binding and dimerization of the Fe–S containing FNR protein from *Escherichia coli* are regulated by oxygen. *J Biol Chem* 271, 2762–2768.

Lee, J. K. & Kaplan, S. (1992). Isolation and characterisation of *trans*-acting mutations involved in oxygen regulation of *puc* operon transcription in *Rhodobacter sphaeroides*. *J Bacteriol* 174, 1158–1171.

Masepohl, B. & Klipp, W. (1996). Organization and regulation of genes encoding the molybdenum nitrogenase and the alternative nitrogenase in *Rhodobacter capsulatus*. *Arch Microbiol* 165, 80–90.

Monson, E. K., Weinstein, M., Ditta, G. S. & Helinski, D. R. (1992). The FixL protein of *Rhizobium meliloti* can be separated into a heme-binding oxygen sensing domain and a functional C-terminal kinase domain. *Proc Natl Acad Sci USA* 89, 4280–4284.

Mosley, C. S., Suzuki, J. Y. & Bauer, C. E. (1994). Identification and molecular characterisation of a sensor histidine kinase responsible for coordinately regulating light harvesting and reaction center gene expression in response to anaerobiosis. *J Bacteriol* 176, 7566–7573.

Neidle, E. L. & Kaplan, S. (1993). Expression of the *Rhodobacter sphaeroides* *hemA* and *hemT* genes, encoding two 5-amino-levalulinic acid synthase enzymes. *J Bacteriol* 175, 2292–2303.

O’Gara, J. P. & 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.

Parkinson, J. S. (1993). Signal transduction schemes of bacteria. *Cell* 73, 857–871.

Pasloske, B. L., Drummond, D. S., Frost, L. S. & Paranchych, W. (1989). The activity of the *Pseudomonas aeruginosa* pilin promoter is enhanced by an upstream regulatory site. *Gene* 81, 25–34.

Pemberton, J. M. & Bowen, A. R. St G. (1981). High frequency chromosome transfer in *Rhodospseudomonas sphaeroides* promoted by the broad host range plasmid RPL carrying the mercury transposon Tn501. *J Bacteriol* 147, 110–117.

Penfold, R. J. & Pemberton, J. M. (1991). A gene from the photosynthetic cluster of *Rhodobacter sphaeroides* induces *trans* suppression of bacteriochlorophyll and carotenoid levels in *R. sphaeroides* and *R. capsulatus*. *Curr Microbiol* 23, 259–263.

Penfold, R. J. & Pemberton, J. M. (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.

Phillips-Jones, M. K. & Hunter, C. N. (1994). Cloning and nucleotide sequence of *regA*, a putative response regulator gene of *Rhodobacter sphaeroides*. *FEMS Microbiol Lett* 116, 269–276.

Ponnampalam, S. N., Buggy, J. J. & Bauer, C. E. (1995). Characterization of an aerobic repressor that coordinately regulates bacteriochlorophyll, carotenoid, and light harvesting-II expression in *Rhodobacter capsulatus*. *J Bacteriol* 177, 2990–2997.

Poole, R. K. (1994). Oxygen reactions with bacterial oxidases and globins: binding, reduction and regulation. *Antonie van Leeuwenhoek* 65, 289–310.

Pratt, L. A. & Silhavy, T. J. (1995). Porin regulon of *Escherichia coli*. In *Two-component Signal Transduction*, pp. 105–127. Edited by J. H. Hoch & T. J. Silhavy. Washington, DC: American Society for Microbiology.

Preisig, O., Zufferey, R. & Hennecke, H. (1996). The *Bradyrhizobium japonicum* *fixGHIS* genes are required for the formation of the high-affinity cbb₃-type cytochrome oxidase. *Arch Microbiol* 165, 297–305.

Qian, Y. & Tabita, F. R. (1996). A global signal transduction system regulates aerobic and anaerobic CO₂ fixation in *Rhodobacter sphaeroides*. *J Bacteriol* 178, 12–18.

Ramakrishnan, G. & Newton, A. (1990). FlbD of *Caulobacter crescentus* is a homologue of the NtrC (NRI) protein and activates σ^{54} -dependent flagellar gene promoters. *Proc Natl Acad Sci USA* 87, 2369–2373.

Richardson, D. J., McEwan, A. G., Jackson, J. B. & Ferguson, S. J. (1989). Electron transport pathways to nitrous oxide in *Rhodobacter* species. *Eur J Biochem* 185, 659–669.

Richardson, D. J., Bell, L. C., McEwan, A. G., Jackson, J. B. & Ferguson, S. J. (1991). Cytochrome *c*₂ is essential for electron transfer to nitrous oxide reductase from physiological substrates in *Rhodobacter capsulatus* and can act as an electron donor *in vitro*. *Eur J Biochem* 199, 677–683.

Schulze, M. & Rodel, G. (1989). Accumulation of the cytochrome *c* oxidase subunits I and II in yeast requires a mitochondrial membrane-associated protein, encoded by the nuclear *sco1* gene. *Mol Gen Genet* 68, 945–954.

Sganga, M. W. & Bauer, C. E. (1992). Regulatory factors controlling photosynthetic reaction center and light-harvesting gene expression in *Rhodobacter capsulatus*. *Cell* 68, 945–954.

Sganga, M. W., Aksamit, R. R., Cantoni, G. L. & Bauer, C. E.

- (1992). Mutational and nucleotide sequence analysis of S-adenosyl-L-homocysteine hydrolase from *Rhodobacter capsulatus*. *Proc Natl Acad Sci USA* **89**, 6328–6332.
- Shimada, H., Iba, K. & Takimiya, K. (1992). Blue light irradiation reduces the expression of *puf* and *puc* operons of *Rhodobacter sphaeroides* under semi-aerobic conditions. *Plant Cell Physiol* **33**, 471–475.
- Shimada, H., Ohta, H., Masuda, T., Shioi, Y. & Takimiya, K. (1993). A putative transcription factor binding to the upstream region of the *puf* operon in *Rhodobacter sphaeroides*. *FEBS Lett* **328**, 41–44.
- Shimada, H., Wada, T., Handa, H., Ohta, H., Mizoguchi, H., Nishimura, K., Masuda, T., Shioi, Y. & Takimiya, K. (1996). A transcription factor with a leucine-zipper motif involved in light-dependent inhibition of expression of the *puf* operon in the photosynthetic bacterium *Rhodobacter sphaeroides*. *J Bacteriol* **178**, 512–522.
- Spiro, S. (1994). The Fnr family of transcriptional regulators. *Antonie van Leeuwenhoek* **66**, 23–36.
- Suwanto, A. & Kaplan, S. (1989). Physical and genetic mapping of the *Rhodobacter sphaeroides* 2.4.1 genome: genome size, fragment identification and gene localization. *J Bacteriol* **171**, 5840–5849.
- Taylor, D. P., Cohen, S. N., Clark, W. G. & Marrs, B. L. (1983). Alignment of genetic and restriction maps of the photosynthesis region of the *Rhodospseudomonas capsulata* chromosome by a conjugation-mediated marker rescue technique. *J Bacteriol* **154**, 580–590.
- Tokito, M. K. & Daldal, F. (1992). *petR*, located upstream of the *fbdFBC* operon encoding the cytochrome bc₁ complex, is homologous to bacterial response regulators and necessary for photosynthetic and respiratory growth of *Rhodobacter capsulatus*. *Mol Microbiol* **6**, 1645–1654.
- Uندن, G., Trageser, M. & Duchene, A. (1990). Effect of positive redox potentials (+400mV) on the expression of anaerobic respiratory enzymes in *Escherichia coli*. *Mol Microbiol* **4**, 315–319.
- van Waasbergen, L. G., Hildebrand, M. & Tebo, B. M. (1996). Identification and characterisation of a gene cluster involved in manganese oxidation by spores of the marine *Bacillus* sp. strain SG-1. *J Bacteriol* **178**, 3517–3530.
- Wellington, C. L., Bauer, C. E. & Beatty, J. T. (1992). Photosynthesis gene superoperons in purple nonsulfur bacteria: the tip of the iceberg? *Can J Microbiol* **38**, 20–27.
- Willison, J. C., Ahombo, G., Chabert, J., Magnin, J. P. & Vignais, P. M. (1985). Genetic mapping of the *Rhodospseudomonas capsulata* chromosome shows non-clustering of genes involved in nitrogen fixation. *J Gen Microbiol* **131**, 3001–3015.
- Yeliseev, A. A. & Kaplan, S. (1995). A sensory transducer homologous to the mammalian peripheral-type benzodiazepine receptor regulates photosynthetic membrane complex formation in *Rhodobacter sphaeroides* 2.4.1. *J Biol Chem* **270**, 21167–21175.
- Yen, H. C. & Marrs, B. L. (1976). Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodospseudomonas capsulata*. *J Bacteriol* **126**, 619–629.
- Yildiz, F. H., Gest, H. & Bauer, C. E. (1991). Genetic analysis of photosynthesis in *Rhodospirillum rubrum*. *J Bacteriol* **173**, 4163–4170.
- Yildiz, F. H., Gest, H. & Bauer, C. E. (1992). Conservation of the photosynthesis gene cluster in *Rhodospirillum rubrum*. *Mol Microbiol* **6**, 2683–2691.
- Zeilstra-Ryalls, J. & 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, J. & Kaplan, S. (1996). Control of *hemA* expression in *Rhodobacter sphaeroides* 2.4.1: regulation through alterations in the cellular redox state. *J Bacteriol* **178**, 985–993.
- Ziegelhoffer, E. C. & Kiley, P. J. (1995). *In vitro* analysis of a constitutively active mutant form of the *Escherichia coli* global transcription factor FNR. *J Mol Biol* **245**, 351–361.