Regulation of photosynthetic gene expression in purple bacteria

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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
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 et al., 1985; Armstrong et al., 1985; Armstrong et al., 1989; Coomber & Hunter, 1989; Bolivar 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 pubA), 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 & Marrs, 1986; Pemberton & Bowen, 1981; Taylor et al., 1983; Willison et al., 1985; Armstrong et al., 1989; Coomber & Hunter, 1989; Bolivar et al., 1994; Fig. 2).

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 Crt− 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 Crt− PpsR− the absence of carotenoids saw maximal photo-oxidative damage. So lethal was photo-oxidative killing of Crt− 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,−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 O2 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 reduce its activity under aerobic conditions. A possible clue comes from the sequence identity between PpsR and the direct oxygen sensor 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 O2 levels directly (Penfold & Pemberton, 1994; Gomelsky & Kaplan, 1995a; J. M. Pemberton & R. J. Penfold, unpublished data).
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 reinhardti, 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 centenarium) 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 *pubA* form a similar 'super operon'. Equally, a superoperonal transcript emanating from *bchF* and terminating beyond *pubA* can be controlled by the PpsR-binding motif of *bchF*. Hence, part of the transcription of the *puf* and *pub* operons can be controlled by PpsR, although neither has a PpsR-binding site controlling their individual operon. However, the major regulator of the *puf* and *pub* 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 *pub* 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* 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 \textit{prrA} in multi-copy can partially suppress the effect of the \textit{prrB} mutation on photosynthetic gene expression (Eraso & Kaplan, 1995). Recently, Qian & Tabita (1996) described a conditional mutant of \textit{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 \textit{reg/prr} genes are transcribed in opposite directions (Fig. 3) and are separated by a third gene known as \textit{senC} in \textit{R. capsulatus} (Bugg & Bauer, 1995) and \textit{prrC} in \textit{R. sphaeroides} (Eraso & Kaplan, 1995). \textit{prrC} is transcribed in the same direction as \textit{prrA} although the details of the transcriptional regulation of the two genes is unknown. The phenotype of a \textit{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 \textit{prrB} or \textit{prrA} was placed in \textit{trans} in a \textit{prrC} null mutant the effect of the mutation was always seen and there appears to be a synergism in the effect of mutations of \textit{prrB} and \textit{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 \textit{Saccharomyces cerevisiae}, \textit{sco1} and \textit{sco2} which are essential for the accumulation of cytochrome oxidase in the mitochondrion (Schulze & Rodel, 1989). SenC has 33\% sequence identity to Sco1 (Bugg & Bauer, 1995) and further sequence analysis revealed that it possesses a sequence motif characteristic of bacterial ferredoxins (Bugg & Bauer, 1995). This certainly suggests that its activity might be influenced by electron transfer reactions, perhaps by components of the photosynthetic and respiratory chain. \textit{prrC} homologues have also been identified in the Mn$^{2+}$-oxidizing gene cluster of a marine \textit{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 \textit{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 \textit{R. sphaeroides}. Tabita and co-workers have shown that mutations in \textit{prrB} blocked transcription of the \textit{cbb} regulon which contains RubisCo genes and other genes of the Calvin-Benson-Bassham (CBB) cycle of CO$_2$ fixation (Qian & Tabita, 1996). Recently, it has been shown that under some circumstances \textit{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 \textit{petR} gene is located upstream of the \textit{fbc} operon (encoding the cytochrome \textit{bc$_1$} complex) and encodes a typical response regulator with a DNA-binding motif (Tokito & Daldal, 1992). Mutations in \textit{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 (\textit{psu}, \textit{bch} and \textit{crt} operons) by antagonizing the action of the repressor PpsR. PpsS (known as ORF192 in \textit{R. capsulatus}) and/or AppA might mediate this process; it is established that a mutation in \textit{orf192} caused \textit{R. capsulatus} to produce decreased amounts of photosynthetic pigments (Bollivar et al., 1994). We have made a similar observation for the \textit{ppss} mutant of \textit{R. sphaeroides} (I. M. Horne, T. A. Williams, A. G. McEwan & J. M. Pemberton, unpublished observations).

**What signals does the Prr system sense?**

\textit{In vitro} autophosphorylation of a truncated form of RegB and transfer of the phosphoryl group to purified RegA from \textit{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 \textit{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 \textit{R. sphaeroides} cells to
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.

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 bc1 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 c5 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 aa3, and a cytochrome cbb3 (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 bc1 complex. However, the two cytochrome oxidases are differentially expressed in response to oxygen tension; the cytochrome aa3 is expressed under highly aerobic conditions while under low oxygen tensions cytochrome cbb3 prevails. A pattern of distribution and expression of cytochrome cbb3 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 $cbb_3$ is interesting because it has recently been found that a mutation in the $eco$ operon (encoding cytochrome $cbb_3$) causes increased synthesis of LH complexes under aerobic conditions (Zeilstra-Rylls & 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 $a_{3}$) there was an increase in the level of LH complex and the expression of $puf$ and $pub$ 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 $cbb_3$. 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 $pub$ promoter regions. Mutation of $hvrA$ resulted in a failure of $R$. *capsulatus* to increase $puf$ and $pub$ 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 $pub$ 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 $abcY$ in response to low light and orfS of unknown function in response to high light. $abcY$ 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. AdoHyc 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/AdoHyc 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 $pub$ 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 $pub$ 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 *Rhodopseudomonas palustris* was identified in 1992 (Dispensa et al., 1992), the identification of an fnr gene in *R. sphaeroides* is more recent (Zeilstra-Rylls & 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-Rylls & 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,
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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 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 (Khoroshlova 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 anaerobic 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 c3) (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.
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 cbb3 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 bc1 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 CuA-binding region of subunit 2 of mitochondrial cytochrome oxidase. Thus, PrR/SenC may contain Cu and not an [Fe–S] cluster. Other factors may influence the insertion of Cu into the CuB site of cytochrome cbb3 in Bradyrhizobium japonicum Fixl (the homologue of Rdxl in R. sphaeroides) has been shown to be required for biogenesis of cytochrome cbb3 (Preisig et al., 1996) Arch Microbiol 165, 297–305]. fixl encodes a P-type ATPase and it has been suggested that it is involved in Cu2+ export. In view of these data it is possible that two pairs of cysteines of RdxB may also bind Cu2+ rather than Fe2+ as suggested in this review. Thus, PrR/SenC and the Rdx systems may influence photosynthetic gene...
expression by affecting the biogenesis of cytochrome $cbb_3$. However, the molecular basis for the modulation of the PrR/Reg sensor regulator and of Fnr by these proteins is still unclear.

References


