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MicroReview

PpsR: a multifaceted regulator of photosynthesis gene expression in purple bacteria

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Summary

Purple bacteria control the level of expression and the composition of their photosystem according to light and redox conditions. This control involves several regulatory systems that have been now well characterized. Among them, the PpsR regulator plays a central role, because it directly or indirectly controls the synthesis of all of the different components of the photosystem. In this review, we report our knowledge of the PpsR protein, highlighting the diversity of its mode of action and focusing on the proteins identified in four model purple bacteria (*Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rubrivivax gelatinosus*, *Bradyrhizobium* ORS278). This regulator exhibits unique regulatory features in each bacterium: it can activate and/or repress the expression of photosynthesis genes, its activity can be modulated or not by the redox conditions, it can interact with other specific regulators and therefore be involved differently in light and/or redox regulatory circuits.

Introduction

The photosynthetic apparatus of purple bacteria consists generally of one or two light harvesting complexes (LH1 and LH2), a photochemical reaction centre (RC) and various electron transport components. This photosystem (PS) allows the bacteria to derive energy from sunlight. However, its functioning in high oxygen tension and at high light intensity could lead to the formation of reactive oxygen species (ROS), in particular singlet oxygen which is highly toxic for the cell. To cope with these dangerous compounds, purple bacteria have developed protective mechanisms using detoxifying enzymes (peroxidase, catalase, superoxide dismutase) or antioxidant activity compounds such as carotenoids, glutathione and thioredoxin (Zeilstra-Ryalls and Kaplan, 2004). In addition, they have elaborated highly sophisticated mechanisms of regulation that control the level and the composition of their PS according to environmental conditions (for review see Oh and Kaplan, 2001; Bauer *et al*., 2003). In this way, the quantity of PS synthesized is affected by oxygen tension, light intensity and light quality.

During the last decade, research conducted mainly by Carl Bauer's and Samuel Kaplan's groups has established the basis of the molecular mechanisms of PS regulation in purple bacteria (Bauer and Bird, 1996; Zeilstra-Ryalls and Kaplan, 2004). Three major regulatory systems have been discovered and characterized: (i) the RegB/RegA (PrrB/PrrA) two-component regulatory system (for review see Elsen *et al*., 2004), (ii) the anaerobic activator FnrL (Zeilstra-Ryalls and Kaplan, 1995; 1998) and (iii) the aerobic repressor PpsR (Penfold and Pemberton, 1994; Ponnampalam *et al*., 1995). In contrast to RegB/RegA and FnrL, which are global regulatory proteins, the PpsR proteins are specially involved in PS regulation.

The PpsR proteins have been mainly characterized in the two closely related species *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. In the latter case, the PpsR counterpart has been historically named CrtJ. For the seek of clarity, the generic nomenclature PpsR will be used in the following, irrespective of the bacterial species. A common mechanism has been unravelled in both bacteria: under oxidizing conditions, PpsR blocks transcription of several photosynthesis gene operons (*bch*, *crt*, *puc*) by binding to the target sequence ($TGTN_{12}ACA$) found in tandem in the promoter regions (Gomelsky and Kaplan, 1995; Ponnampalam and Bauer, 1997). The for-

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mation of an intramolecular disulphide bond has been shown to stimulate the binding of the two PpsR proteins to their target promoters (Masuda and Bauer, 2002; Masuda *et al*., 2002). Nevertheless, contrary to PpsR from *R. capsulatus*, the repressive activity of *R. sphaeroides* PpsR is antagonized by another regulator, AppA, which integrates both light intensity and redox signals (Gomelsky and Kaplan, 1997; Masuda and Bauer, 2002; Braatsch *et al*., 2002). The regulator AppA has been identified only in *R. sphaeroides,* suggesting the existence of a specific regulatory mechanism of PS synthesis in this bacterium. Furthermore, recent studies using other model bacteria (*Rubrivivax gelatinosus* and *Bradyrhizobium* ORS278) have also revealed distinctive mechanisms of regulation involving PpsR. In particular, *R. gelatinosus* PpsR can act both as an activator and as a repressor, depending on the photosynthesis genes (Steunou *et al*., 2004). *Bradyrhizobium* has two PpsR proteins with antagonistic roles on the same photosynthesis genes. In addition, one of these PpsRs is not redox sensitive but its activity seems to be modulated by light via a bacteriophytochrome (Giraud *et al*., 2002; Jaubert *et al*., 2004). Altogether, these data suggest that each species uses a specific strategy to regulate PS formation via a diversity of action of its regulator PpsR.

The aim of this review is to highlight the common and specific behaviours of the conserved regulator PpsR in different purple bacteria.

Genetic organization

In all the purple bacteria in which it has been discovered, the *ppsR* gene has always been found in the photosynthesis gene cluster (PGC). This region of about 45 kb contains the main genes implicated in the synthesis of the PS: the *bch* and *crt* genes involved in the synthesis of bacteriochlorophyll and carotenoid photopigments, respectively, the *pucBAC* and *pufBA* operons encoding the light-harvesting polypeptides, the *puhA* and *pufLM* genes encoding the RC subunits, and other regulatory genes as *tspO* and *aerR* (Alberti *et al*., 1995; Choudhary and Kaplan, 2000). Unexpectedly, *Bradyrhizobium* sp. ORS278 strain and *Rhodopseudomonas palustris* have two *ppsR* genes with a conserved genetic arrangement in the PGC (see Fig. 1) (Jaubert *et al*., 2004). As the two genes in the same bacterium exhibit only low amino acid sequence identity (around 32%), they do not result from a recent duplication event.

A blast search using PpsR sequences indicates that this transcription factor is specific to purple bacteria. Furthermore, a sequence alignment of the nine available sequences of PpsR shows that this family of regulators is not well conserved, as the percentage of identity ranges from 26% to 54%. This high level of divergence could reflect a high rate of evolution of PpsRs and possible variations of their intrinsic properties.

Structure and biochemical properties

Although the various *ppsR* genes display low sequence similarities, the architecture of the corresponding proteins is generally conserved, comprising three different regions. PpsR possesses a C-terminal Helix-Turn-Helix (HTH) motif, whose key role in DNA binding has been confirmed by the study of Gomelsky *et al*. (2000). A particular case is the PpsR protein from *Roseateles depolymerans* whose sequence does not show any HTH signature (Suyama *et al*., 2002), raising the question of the functionality of this protein.

The N-terminal region, which represents almost a third of the protein, does not present obvious similarity with any known domains. However a Per-Arnt-Sim (PAS) domain can be identified in some proteins, as in PpsR from *R. gelatinosus* (Steunou *et al*., 2004) and from *Rhodospirillum rubrum*, and PpsR2 from *R. palustris*. Furthermore, we have identified in most of the proteins a coiled-coil glutamine rich segment, that was earlier reported as a Q-linker (Gomelsky *et al*., 2000), at the end of this N-terminal part. This motif has been identified as the dimerization determinant for several transcription factors (Lupas, 1996) and could play therefore a key role in the oligomerization state of PpsR. An analysis of various spontaneous *ppsR* mutations in *R. sphaeroides* has revealed that this N-terminal part of the protein is essential for the integrity of the protein although its function remains unknown (Gomelsky *et al*., 2000).

The central region of PpsR contains two PAS domains, that were reported to be critical for proper conformation and repressor activity of the protein (Gomelsky *et al*., 2000). The PAS domains are found in proteins from Bacteria, Archaea and Eucarya and are involved in signal sensing and transduction (for review see Taylor and Zhulin, 1999). Another possible role of PAS domains is in protein-protein interactions. In many cases, these domains bind specific cofactors (flavin, haem, or metals) that respond to changes in environmental conditions. To date, none of the PpsR proteins purified after overproduction in *Escherichia coli* was found to contain any cofactors, suggesting that the redox-dependent DNA binding response observed for most of them results from an intrinsic property. In agreement with this proposal, recent biochemical studies of PpsR from *R. capsulatus* or *R. sphaeroides* have clearly shown the critical role of an intramolecular disulphide bond for the binding of the proteins to their target promoters (Masuda and Bauer, 2002; Masuda *et al*., 2002). Two cysteine residues that are conserved in the two proteins (C251 and C424 in *R. sphaeroides* PpsR and C249 and C420 in *R. capsulatus* PpsR)

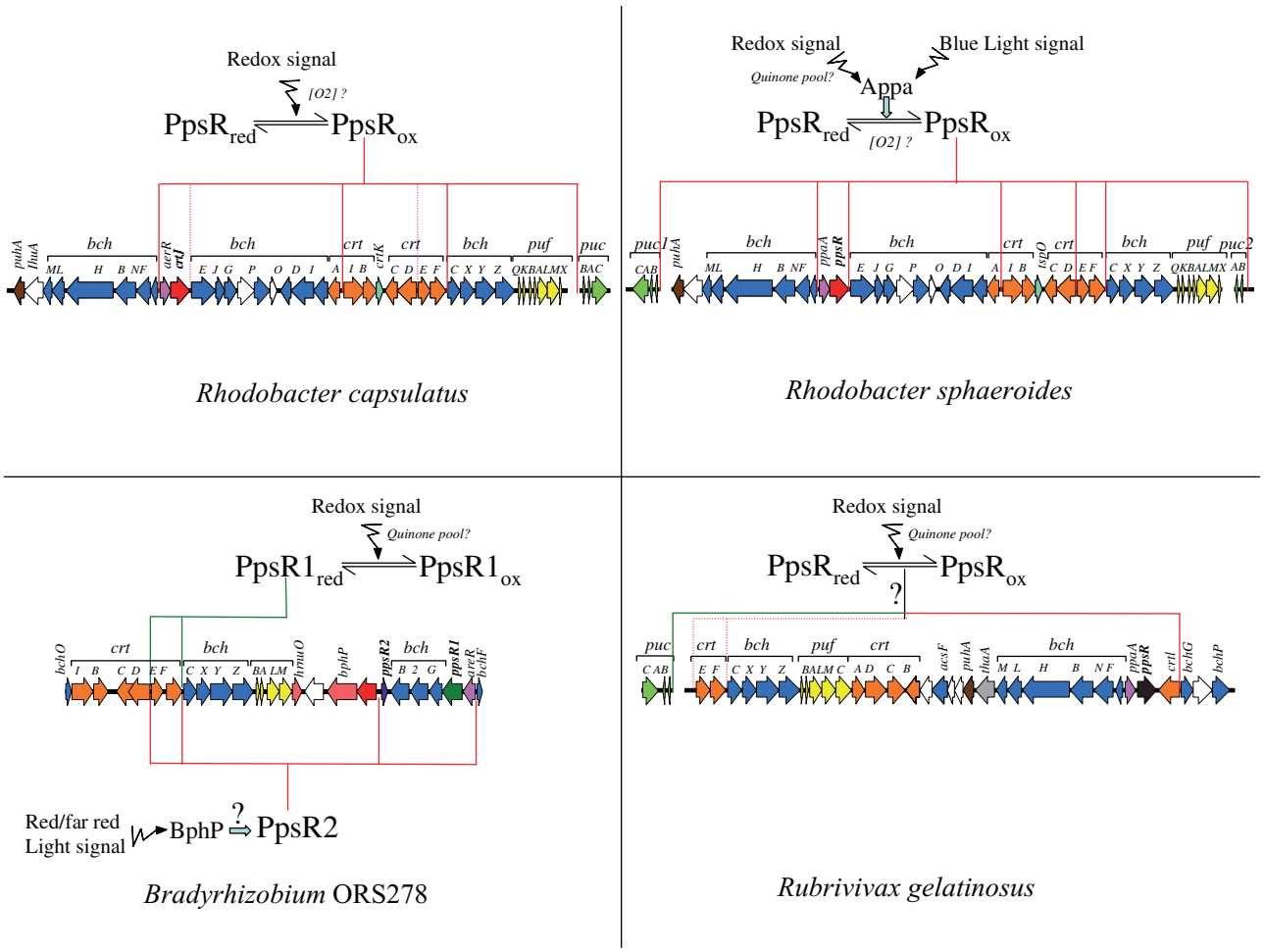

Fig. 1. Diagram of the various regulatory circuits involving PpsR in the control of photosynthesis gene expression characterized in *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, *Rubrivivax gelatinosus* and *Bradyrhizobium* sp. ORS278. The relative arrangement of the genes belonging to the photosynthesis gene clusters (PGC) is indicated for each bacterium. The *puc* operons encoding the light harvesting-II structural polypeptides are not linked to the PGC. Red and green lines indicate a negative and a positive control exerted directly by PpsR respectively. Dashed lines indicate the putative action of PpsR deduced from the sequence analysis of the promoter regions. The question marks close to O_2 and the quinone pool indicate their possible effect on the modulation of redox state of PpsR. The question mark between BphP and PpsR2 raises the question of a direct or an indirect interaction between these two regulators, whereas that in the *R. gelatinosus* box questions the redox state of PpsR that binds to the DNA.

were shown by mutagenesis to be the redox-sensing residues (Gomelsky *et al.*, 2000; Masuda and Bauer, 2002; Masuda *et al.*, 2002). However, a comparison with the other PpsR sequences indicates that only the Cys residue located in the HTH domain is well conserved. The change of redox state of its thiol group into diverse possible derivatives (sulphenic acid, sulphinic acid, sulphonic acid or disulphide bond) is likely a general scheme for modulating the DNA binding affinity of most PpsRs.

Among the different PpsR proteins characterized, two other cases are particularly informative in the overall understanding of the redox response of PpsR. First, PpsR1 of *Bradyrhizobium* has only one Cys residue (in the HTH domain), and second, the PpsR2 proteins of both *Bradyrhizobium* and *R. palustris* do not contain any Cys residue. In the first case, PpsR1, in spite of its unique Cys residue, retains redox sensitivity via the formation of an intermolecular disulphide bond (Jaubert *et al.*, 2004). Interestingly, contrary to the *R. capsulatus* and *R. sphaeroides* PpsR proteins, which are both stable tetramers irrespective of the redox conditions, the formation of an intermolecular disulphide bond in PpsR1 leads to a global change of its quartenary structure, switching it from a tetramer to an octamer. In the case of PpsR2, no direct redox effect has been observed on its DNA binding activity (Jaubert *et al.*, 2004). This is in complete agreement with the critical role played by Cys residue(s) in redox sensing by the other PpsRs.

While we can assume from all these data that most of the PpsR proteins sense a redox signal via one or two

critical Cys residue(s), the signal modulating their redox state *in vivo* remains unclear. In *R. sphaeroides* conflicting results were reported concerning the *in vivo* redox state of PpsR. Indeed, Cho *et al*. (2004) observed that the two Cys residues in *R. sphaeroides* PpsR are reduced whatever the growth conditions. On the contrary, Masuda and Bauer (2002) observed the formation of an intramolecular bond specifically under aerobic culture condition. In addition, Bauer's group has shown that the midpoint redox potential of the cytosol in *R. capsulatus* is around −220 mV under both aerobic and anaerobic conditions, a value lower than the midpoint potential estimated for oxidation of PpsR (−180 mV) (Masuda *et al*., 2002). This leads the authors to suggest that PpsR is oxidized directly by the presence of molecular oxygen in accordance with their observations that PpsR forms a disulphide bond *in vivo* and *in vitro* when exposed to oxygen. These discrepancies may reflect the difficulties in estimating the cytosolic redox potential or in blocking the redox state of PpsR during its extraction. Therefore, we cannot exclude that *R. capsulatus* PpsR responds to another redox signal than oxygen, and further experiments are necessary to clarify this point.

One interesting hypothesis proposed in the case of *R. sphaeroides* is that PpsR senses the redox state of the quinone pool via the flavoprotein AppA (Oh and Kaplan, 2000). AppA is an essential actor of PS synthesis in *R. sphaeroides*, not found in the other purple bacteria, that was shown to modulate the repressive activity of PpsR *in vivo* (Gomelsky and Kaplan, 1997) (see section 'Involvement of PpsR in light regulatory circuits'). This hypothesis is particularly appealing because the quinone pool acts as a branching point between electron carriers of the photosynthetic and respiratory chains, i.e. the photosystem itself, terminal oxidases as aa_3- and cbb_3-type cytochrome *c* oxidases, quinol oxidases, DMSO reductase. Therefore, the redox state of the quinone pool depends on various environmental factors, including light intensity, oxygen consumption, available substrates, CO_2 and N_2 fixation, etc. The assumption made by Oh and Kaplan (2000) relies on several findings that we have briefly summarized (for further information, see the reviews Oh and Kaplan, 2001; and Zeilstra-Ryalls and Kaplan, 2004): (i) the decrease, observed under anaerobic condition, of photosynthesis genes expression, in particular of the *puf* and *puc* genes, after the addition of external electron acceptors such as DMSO oxidizing the quinone pool (Horne *et al*., 1996; Oh and Kaplan, 2000). This indicates that this expression is directly coupled to the redox state of the quinone pool, (ii) the repression of PS synthesis in an *appA* null mutant, even under anaerobic condition, suggesting that the repressive effect of PpsR is not directly affected by redox change (Gomelsky and Kaplan, 1997), and (iii) the ability of AppA to reduce *in vitro* PpsR (Masuda and Bauer, 2002) and (iv) AppA is a good potential sensor of the redox state of the quinone pool because it contains one FAD binding domain and a putative haem-binding domain (Gomelsky and Kaplan, 1998; Oh and Kaplan, 2001). However, if this series of argumentation seems well coherent and gives weight to the assumption made by Oh and Kaplan, it is to note that no direct proof has been given yet.

The mechanism modulating the redox state of *Bradyrhizobium* PpsR1 also remains elusive, as air has no effect on the purified protein, suggesting that the protein is oxidized *in vivo* by a stimulus other than oxygen (Jaubert *et al*., 2004). Could the quinone pool transduce a redox signal to the protein via another partner? Further studies are required to determine the molecular mechanisms controlling the redox state of PpsR in purple bacteria.

DNA binding properties

A common feature of all the PpsR proteins studied so far is that they share the same DNA binding site: the palindromic sequence $TGTN_{12}ACA$. A recent survey of the *R. sphaeroides* genome identified 240 copies of this palindrome (Moskvin *et al*., 2005). However global transcriptome analysis of the PpsR regulon revealed that two palindrome sites are required for an effective *in vivo* repression by PpsR, which substantially reduced the number of potential target genes (Moskvin *et al*., 2005). This is in accordance with previous *in vitro* gel mobility shift and DNase I footprinting studies demonstrating that *R. capsulatus* PpsR binds to two adjacent palindromes in a cooperative manner (Ponnampalam and Bauer, 1997). The two PpsR binding sites are generally spaced by 7 or 8 base pairs (bp), and overlap the −35 and −10 motifs of the σ^{70}-type promoters, suggesting competition between PpsR and RNA polymerase (RNAP) for binding. The spacing between the two palindromes is crucial for cooperative binding, as increasing the spacing by 6 or 11 bp (half-helical or full-helical insertion) abolished PpsR binding (Ponnampalam *et al*., 1998). Cooperative interactions have also been found with *R. sphaeroides* PpsR (Masuda and Bauer, 2002) and with *Bradyrhizobium* PpsR1 and PpsR2 (Jaubert *et al*., 2004). This might be a conserved mechanism of DNA binding among the PpsR proteins, allowing them to switch gene transcription off (or on, see below) through a slight variation in the amount of active and inactive protein. Nevertheless, the quaternary form that interacts with DNA is still debated. Studies with *R. capsulatus* PpsR led Ponnampalam and Bauer (1997) to propose that the dimeric protein binds to one palindrome and that two dimers cooperatively interact to form a tetramer. As a result, each monomer would interact with each palindrome half site. More recent models, based on the observations that PpsR proteins exist as stable tetra-

mers in solution, depicted the binding of a tetramer to the two palindromes (Masuda and Bauer, 2002; Masuda *et al*., 2002). However the data we obtained with PpsR from *Bradyrhizobium* led us to propose the cooperative binding of two PpsR tetramers to the adjacent palindromes in order to explain the cooperative binding effect (Jaubert *et al*., 2004). The way the proteins interact with the DNA binding sites is still under investigation.

A second class of PpsR-regulated promoters corresponding to two distant palindromes has also been described (for example, up to 240 bp separate the two palindromes found in the *pucBA* promoter of *R. capsulatus*) (Elsen *et al*., 1998). Interestingly, PpsR from *R. capsulatus* exerts a similar level of repression on the promoters harbouring distantly spaced or neighbouring palindromes. Moreover, the fixation is also cooperative and presumably involves the formation of a DNA loop (Elsen *et al*., 1998). In the case of these promoters, other regulatory proteins could also bind between the two palindromes and integrate other stimuli. Indeed, it has been demonstrated that AerR, another repressor protein, can bind to the *crtI-crtA* intergenic sequence and stimulate PpsR binding, probably by bending the DNA (Dong *et al*., 2002). The integration host factor (IHF), another DNA-bending protein, could also play a role, because putative binding sites have been found within the same intergenic sequence, as well as in the *puc* promoter (Nickens and Bauer, 1998; Elsen *et al*., 1998).

Based on the promoter sequences of PpsR-regulated genes that were identified by transcriptome analysis in *R. sphaeroides*, Moskvin *et al*. (2005) proposed a refined consensus sequence for the PpsR binding site ($TGTcN_{10}gACA$). We have compared the identified PpsR binding sites from the purple bacterium models discussed in this review (*R. capsulatus, R. sphaeroides, R. gelatinosus, R. palustris and Bradyrhizobium* ORS278). The results (Fig. 2) are slightly different from those obtained using *R. sphaeroides* alone, as less weight is given to the two nucleotides C and G found contiguous to the motifs TGT and ACA. In contrast, the adjacent nucleotides A and T are strongly conserved, suggesting that they also play a critical role in the efficient binding of the PpsR proteins. We propose that the consensus PpsR binding site is $TGTcAN_8TgACA$. Some variations in the palindromic sequences are possible, except for the G and C nucleotides that are strictly conserved in all of them. In agreement with this proposal, PpsR from *R. capsulatus* binds with the same affinity to the conserved $TGTN_{12}ACA$ palindrome and to the second $TGTN_{12}ACG$ palindrome in the divergently transcribed *crtI* and *crtA* genes of *R. capsulatus* (Elsen *et al*., 1998). While these data indicate that a slight variation is possible in the recognized DNA regions, a recent study reveals that the PpsR proteins can differ in their ability to bind to different sites. Indeed, only PpsR2 of *Bradyrhizobium* is able to bind *in vitro* to the *cycA* and *aerR* promoters. This was unexpected because PpsR1 and PpsR2 exhibit the same DNA binding efficiency to the *bchC* and *crtED* promoters (Jaubert *et al*., 2004). The difference between these two types of promoters resides in the sequence conservation of the two DNA binding sites: they are both perfectly conserved in the *bchC* and *crtED* promoters, whereas only one conserved binding site is found together with an incompletely conserved one in the *cycA* and *aerR* promoters. Furthermore, efficient binding of PpsR2 has only been observed on the conserved palindrome (Jaubert *et al*., 2004). This raises the question of the putative role of the non-conserved palindrome in the DNA binding mechanism of PpsR2 and opens up the possibility that, contrary to dogma, some PpsR proteins can regulate gene expression by binding to promoters harbouring a single palindrome. A consequence of this observation is that the two PpsR proteins in *Bradyrhizobium* should affect different panels of target genes.

Usually, the DNA binding activity of the PpsR proteins is redox controlled by formation/disruption of disulphide bonds within the proteins, as already described. The effect of the redox potential on the DNA binding affinity was first shown with *R. capsulatus* PpsR. In accordance with its role as an aerobic repressor (Ponnampalam *et al*., 1995), Ponnampalam and Bauer (1997) observed by gel mobility shift assays that PpsR binds to DNA more tightly (four- to fivefold) under oxidizing- than under reducing conditions. A similar behaviour was observed with PpsR from *R. sphaeroides*, but with only a twofold difference in binding activity. This difference, which could appear insufficient for properly regulating gene expression *in vivo*, is increased up to fivefold via the action of the flavoprotein AppA, which exerts an additional control level on the DNA binding activity of PpsR (Masuda and Bauer, 2002) (see below). How-

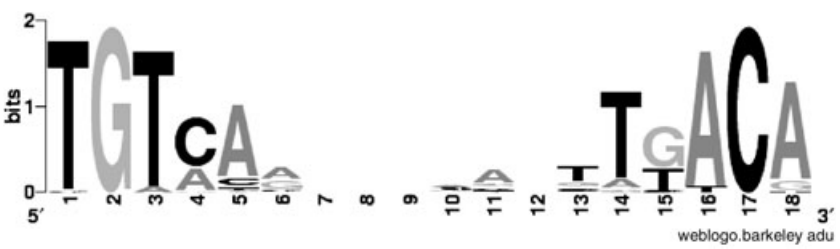

Fig. 2. Sequence motif of the consensus sequence for PpsR binding sites identified in *R. capsulatus*, *R. sphaeroides*, *R. gelatinosus* and *Bradyrhizobium* sp. ORS278 (Crooks *et al*., 2004; http://weblogo.berkeley.edu).

ever, Cho *et al*. (2004) have challenged this model following their observation that the DNA binding affinity of *R. sphaeroides* PpsR increases *in vitro* under reducing conditions. This observation is in complete contrast to the previous studies of Bauer's group and does not seem consistent with the aerobic repressive role proposed for PpsR. Further investigations are required to clarify these puzzling data. Interestingly, a recent study with *Bradyrhizobium* has also revealed that the affinity of PpsR1 for its DNA target is a fourfold higher in its reduced form compared with its oxidized form (Jaubert *et al*., 2004). As genetic analyses suggest that PpsR1 plays an activating role under semiaerobic conditions, this is in total agreement with its redox-binding feature (Jaubert *et al*., 2004).

Hence, it appears that the DNA binding mechanism of the PpsR proteins is far more complex than initially thought, as proteins from various purple bacteria as well as the two proteins from the same bacterium behave differently.

Different modes of action: repressor/activator/dual activities

For more than a decade, PpsR was considered to be a repressor. The position of its DNA binding sites, either overlapping the −10 and −35 regions or just upstream from the −35 motif of its target σ-70 promoters, suggests that PpsR acts by preventing the RNAP from binding to its DNA sites. Indeed, using *in vitro* transcription assays with *R. capsulatus* housekeeping RNAP, Bowman *et al*. (1999) demonstrated that PpsR represses *bchC* gene transcription by competing with RNAP for binding to DNA. However, they also discovered another mechanism of repression on the *puc* promoter, on which PpsR can compete with the activator RegA protein, preventing RegA-mediated activation.

Unexpectedly, the homologous PpsR protein in *R. gelatinosus* was reported to be a «dual regulator» being able to repress the *crtI* gene and to activate *pucB* (Steunou *et al*., 2004). Both PpsR DNA binding sites overlap the putative −10 and −35 sequences but are separated by 9 bp in the *puc* promoter and by 8 bp in the *crtI* promoter (Steunou *et al*., 2004). The authors proposed that the behaviour of PpsR (activator/repressor) could be related to this difference in spacing between the two palindromes (Steunou *et al*., 2004). However, we wish to point out that a difference of 1 bp in the spacing of the DNA binding sites of *R. sphaeroides* PpsR (8 bp on *bchC* promoter vs. 7 bp on *puc* promoter operons; Ponnampalam *et al*., 1995; Zeng *et al*., 2003) does not affect the repressor role of the protein.

In *Bradyrhizobium* ORS278, the duality was recently shown to result from the presence of two proteins, each possessing a single activity: PpsR1 is an activator whereas PpsR2 is a repressor (see Fig. 1) (Giraud *et al*., 2002; Jaubert *et al*., 2004). Interestingly, both proteins bind to the same DNA region (two conserved palindromes separated by 7 bp). In this case, the difference in transcriptional activity is clearly not related to a difference in spacing between the two palindromes. As the DNA binding sites are located within the −10 and −35 region (Jaubert *et al*., 2004), the two PpsR proteins could differently affect transcription, either positively (recruitment of RNAP) or negatively (exclusion of RNAP), possibilities that need to be further studied. Structure-function investigation of the two PpsR proteins might highlight a role for the two PAS domains (which are different in the two proteins) in the different mechanisms of transcription control. It also needs to be determined whether the two PpsR proteins identified in *R. palustris* behave in the same way as those of *Bradyrhizobium*.

Recently, Smart *et al*. (2004) found that inactivation of *R. capsulatus* PpsR led to decreased expression of the *hemE* and *hemH* genes in semiaerobiosis. This suggests that PpsR is a dual regulator in *R. capsulatus* as well, in agreement with the report of Madan Babu and Teichmann (2003) on the position of the DNA binding sites and domain organization of numerous transcription factors. Indeed, this study revealed that, whereas the DNA binding domains are consistently found at the N-terminus of repressor proteins, they are either located in the N-terminal or C-terminal region of activator proteins or dual regulators. The DNA-binding domain of PpsR is C-terminal.

The PpsR regulon

In all the organisms studied so far, the genes directly controlled by the PpsR proteins are consistently photosynthesis genes (the genes involved in the biosynthesis of the bacteriochlorophyll (*bch*) and carotenoid (*crt*) photopigments as well as those encoding the structural light-harvesting II (*puc*) (Fig. 1). A striking difference between *R. capsulatus* and *R. sphaeroides* is the involvement of PpsR in the expression of the *aerR* gene (or its homologue *ppaA* in *R. sphaeroides*). *R. capsulatus* PpsR does not affect *aerR* expression (Dong *et al*., 2002) but PpsR of *R. sphaeroides* directly represses *ppaA* gene transcription (Gomelsky *et al*., 2003). Interestingly, although the AerR/PpaA proteins are present in all anoxygenic phototrophic proteobacteria (Gomelsky *et al*., 2003), their role is quite different depending on the bacteria. Indeed, *R. capsulatus* AerR is a DNA-binding protein that acts as a second aerobic repressor of some photosynthesis genes, whereas *R. sphaeroides* PpaA has been reported to activate photopigment production and *puc* operon expression under aerobic conditions (Gomelsky *et al*., 2003). As it might bind a corrinoid cofactor, PpaA might sense the

integrity of the vitamin B12 biosynthetic pathway that is required for correct PS synthesis (Gomelsky *et al*., 2003). This control of PpaA synthesis by PpsR could be of great importance because, by controlling expression of another regulatory protein, *R. sphaeroides* PpsR would broaden its set of target genes. Jaubert *et al*. (2004) reported that *Bradyrhizobium* PpsR also regulates expression of PS transcription factors; indeed DNA footprinting and gene expression analyses showed that the light-regulated PpsR2 protein regulates not only the synthesis of AerR but also that of the redox-regulated PpsR1 protein by binding to the promoter of a putative *aerR-ppsR1* operon, with all the regulatory consequences we can expect from this finding.

Interestingly, it was reported that *R. capsulatus* PpsR also controls *puf* expression, although no PpsR DNA-binding sites were found in the promoter of this operon (Abada *et al*., 2002). A possible explanation was that this control exerted by PpsR might result from the organization of the photosynthesis genes in so-called superoperons (Wellington and Beatty, 1991; Bauer *et al*., 1991; Beatty, 1995). However, this can be excluded as the *puf* promoter was also affected by PpsR when carried on a plasmid without any upstream genes (Abada *et al*., 2002). A whole genome transcriptome analysis of the PpsR regulon in *R. sphaeroides* also revealed that not only PpsR affects expression of the *puf* operon, but also of the *puhA* operon (Moskvin *et al*., 2005). The absence of DNA-binding sites on these promoters strongly suggests that the regulatory effect of PpsR on these PS genes is surely an indirect effect and implies that the protein controls the synthesis (or activity) of another transcriptional regulator (Moskvin *et al*., 2005). This is supported by the observation that, after blue light exposure, the repressive effect of PpsR on these genes is delayed compared with that on the known target genes, suggesting that a lag period is required to control the level of another regulator (Braatsch *et al*., 2004; Moskvin *et al*., 2005). This regulator might be the activator PpaA, whose synthesis is controlled by PpsR, although Moskvin *et al*. (2005) suggested that it could also be the global response regulator PrrA, because transcriptome analysis reveals that *prrA* expression depends on PpsR levels. This is quite surprising, as the *prrA* gene expression was reported to be independent of oxygen levels in *R. sphaeroides* (Roh *et al*., 2004), and mutation in *ppsR* does not affect the entire PrrAB regulon. Therefore, even if some clues are emerging, the exact mechanism by which PpsR indirectly controls the *puh* and *puf* operons is unclear and needs to be deciphered.

Besides the control of PS synthesis, PpsR regulates the expression of a limited number of non-photosynthesis genes, as revealed by the *R. sphaeroides* PpsR transcriptome analysis (Moskvin *et al*., 2005). It has been shown that PpsR directly represses the expression of two divergently transcribed genes, *hemC* and *hemE*, which code for two enzymes of the tetrapyrrole biosynthetic pathway. A role for *R. capsulatus* PpsR in the regulation of several *hem* genes was already reported by Smart *et al*. (2004). This regulation, made in concert with AerR, would be both direct and indirect, and is quite complex, because PpsR displays both a negative and a positive role, as a function of the target promoters and the oxygen tensions. The control exerted by PpsR on the *hem* genes was reported to be quite substantial and higher than that observed on the photosynthesis genes, which stresses the importance of PpsR in *hem* gene regulation (Smart *et al*., 2004). This protein was also found to be involved in the regulation of the *cydAB* genes coding for an ubiquinol oxidase but this is likely to be an indirect effect as no PpsR DNA binding sites are observed in the corresponding promoter (Swem and Bauer, 2002). Another important observation is the control of the *cycA* gene in *Bradyrhizobium* ORS278: PpsR2, but not PpsR1, has been shown to bind to its promoter *in vitro* (Jaubert *et al*., 2004). The *cycA* gene codes for a cytochrome c_2 that is required for both photosynthesis and respiration. Even if it is not considered in a strict sense as a 'photosynthesis' gene, it is to note that the gene is located within the PGC in some purple bacteria (Fig. 1).

In conclusion, the PpsR regulon differs among the various purple bacteria. However, in every case, the PpsR protein plays a central role in the control of PS synthesis as it regulates most of the photosynthesis genes (*bch, crt, puc,* as well as *puf* and *puh* in *R. sphaeroides*). In addition, as a network architect, it can regulate other essential PS regulators such as AerR/PpaA and PpsR1 in order to coordinate their activities and therefore orchestrate the synthesis of all of the different components of the PS.

Involvement of PpsR in light regulatory circuits

In *R. sphaeroides*, PpsR is clearly involved not only in the redox regulation of photosynthesis genes, but also in their repression in response to high light, although light does not directly affect PpsR DNA binding activity (Gomelsky and Kaplan, 1997). Genetic and biochemical experiments demonstrated that its interaction with another protein, AppA, confers on PpsR the ability to integrate the two stimuli, redox and light intensity. The system was called the AppA/PpsR antirepressor/repressor system (Masuda and Bauer, 2002; Braatsch *et al*., 2002). AppA is a blue-light photoreceptor with several structural features such as a N-terminal BLUF domain with a non-covalently bound FAD and a Cys-rich motif in the C-terminal domain (Gomelsky and Kaplan, 1998; Gomelsky and Klug, 2002). This flavoprotein exhibits a photocycle whose photochemistry has been studied, and senses blue light via its FAD (Masuda and Bauer, 2002; Braatsch *et al*., 2002; Laan

et al., 2004). AppA is able to catalyse disulphide bond reduction in PpsR in a light-independent manner, and can form an AppA-$PpsR_2$ complex that is inhibited by blue-light (Masuda and Bauer, 2002). It specifically modulates the activity of PpsR, as shown by the similar whole-genome transcriptional profiles of an *appA* mutant and a strain overexpressing *ppsR* (Braatsch *et al*., 2004). AppA has also been reported to contain a haem-binding domain (Oh and Kaplan, 2001) that could bind either a haem or a tetrapyrrole intermediate. By binding this coeffector molecule, the protein would link the regulation of photosynthesis genes to tetrapyrrole biosynthesis (Zeilstra-Ryalls and Kaplan, 2004). That would explain how TspO, which is an outer membrane protein involved in efflux of a tetrapyrrole intermediate, could be a modulator of the AppA/PpsR system, as observed (Zeng and Kaplan, 2001).

In *Bradyrhizobium* as well as in *R. palustris*, the absence of Cys residue in the PpsR2 proteins pointed to a mechanism for modulating their activity that differs from the «classical» disulphide bond formation in response to redox (Jaubert *et al*., 2004). In both bacteria, the gene adjacent to *ppsR2* encodes the bacteriophytochrome BphP that is absolutely required for PS synthesis (Giraud *et al*., 2002; 2004), suggesting a functional link between the *ppsR2* and *bphP* genes. Indeed, inactivation of *ppsR2* and the *ppsR2-bphP* genes led to the same phenotype: constitutive PS synthesis in semiaerobiosis, irrespective of the light conditions. Photoconversion of BphP between a red-light absorbing form (Pr) and a far-red-light absorbing form (Pfr) has been demonstrated, with the Pr form being active and involved in the stimulatory effect of PS synthesis by far-red light. Therefore, BphP would be upstream in the regulatory cascade, with the Pr form antagonizing the repressive effect of PpsR2. Unlike other bacteriophytochromes, the BphP proteins do not have a C-terminal histidine kinase domain, implying a light signalling pathway that is different from the classical phosphorelay observed for most bacteriophytochromes (Bhoo *et al*., 2001). It is tempting therefore to suggest that they could directly interact with PpsR2, thus forming a light-dependent BphP/PpsR2 complex, as observed in *R. sphaeroides* with the AppA/PpsR complex. Nevertheless, direct interaction between BphP and PpsR2 has not been shown so far (E. Giraud, unpubl.), and it is likely that another protein is involved in the complex formation.

From these two examples, it appears that the implication of PpsR in the light regulatory circuit does not result from its intrinsic properties but, rather, from its direct or indirect interaction with a light sensor partner. Interestingly, the nature of this partner can be fundamentally different; in the first case, AppA is a blue light sensor whose response depends on light intensity, whereas BphP is a red/far-red light sensor that responds mainly to light quality. This leads to different effects of the light on PS formation in these two bacteria; the synthesis of PS in *R. sphaeroides* is limited under high light intensity, thereby protecting the cell from the generation of ROS, whereas PS synthesis in *Bradyrhizobium* is triggered only by light of wavelength ranging from 700 nm to 770 nm (Giraud *et al*., 2002). It is proposed in the later case that this particular mechanism of regulation via BphP would permit PS synthesis to be increased during the interaction of the bacteria with the plant, specifically in the stem nodules where the bacteria grow below a layer of plant cells containing chlorophyll that preferentially absorb blue and red light but transmit far-red light (for review see Giraud & Fleishman 2004). The interaction of PpsR with different light regulators has surely allowed each bacterium to adapt specifically the PS synthesis to its particular light environment.

Concluding remarks

The recent characterization of the PpsR proteins from various purple bacteria has broadened our view that this regulator family acts simply as an aerobic repressor of some photosynthesis genes. It appears from the few examples discussed here that purple bacteria use different strategies to regulate their PS via their common regulator PpsR that can be implicated differently in light and redox regulatory circuits and can act as a repressor and/or an activator.

Very recently, a global transcriptome analysis of *R. sphaeroides* has confirmed the role of this regulator in the control of PS formation. Indeed, most of the photosynthesis genes have been shown to be regulated by PpsR, either directly or indirectly, including even genes encoding other PS regulators, which led Gomelsky's group to qualify PpsR as a 'master regulator of PS' (Moskvin *et al*., 2005). Interestingly this analysis has also revealed that PpsR activity is mainly limited to the photosynthesis genes. The localization of the *ppsR* gene within the PGC and the absence of homologous genes in other bacteria are additional arguments pointing to the specialization of PpsR in the control of photosynthesis genes. This is a striking difference with the RegB/RegA (PrrB/PrrA) system, the second main regulator of PS in the purple bacteria. Indeed, the *regA/regB* genes are located in another region of the genome and homologous genes are found in a wide variety of photosynthetic and non-photosynthetic bacteria. Furthermore, besides the control of photosynthesis, the RegB/RegA system is also involved in the regulation of several other cellular processes as CO_2 fixation, N_2 assimilation, hydrogen utilization, denitrification, aerotaxis, electron transport (reviewed in Elsen *et al*., 2004). Because it is subject to fewer constraints, PpsR has probably benefited from a higher evolution rate and therefore a greater flexibility than the RegB/RegA system whose

implication in so many fundamental metabolisms could have limited its evolution.

Purple bacteria are widely distributed in nature and are found in all kind of aquatic environments (lakes, marine and hyper saline environments, waste water, coastal lagoons, paddy field, mud, etc.). They are therefore subject to different selective pressures of light and oxygen conditions. We hypothesize that, thanks to the flexibility of their master PS regulator (PpsR), the regulation of the PS has been adapted specifically to their particular ecological niche, allowing them to reach a balance between the benefit of gratis energy and the problems that could result from the generation of ROS.

In the future, we expect that the characterization of other purple bacteria isolated from different environments will lead to the discovery of new biochemical properties, new partners and new regulatory mechanisms of the PpsR regulator.

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