Interacting regulatory networks in the facultative photosynthetic bacterium, *Rhodobacter sphaeroides* 2.4.1

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Abstract

Regulation of photosynthetic membrane synthesis in *Rhodobacter sphaeroides* 2.4.1 is dependent on the interactions of numerous regulatory elements, with two of the most important being the *cbb*$_3$ terminal oxidase and the PrrBAC two-component regulatory system. Here, we reveal that the *cbb*$_3$ terminal oxidase possesses extensive, additional regulatory activities under anaerobic conditions, and that the PrrBAC system is further involved in the regulation of the expression of more than 20% of the *R. sphaeroides* genome under anaerobic conditions, extending well beyond functions related to redox gene expression.

*Rhodobacter sphaeroides* is a Gram−-, non-sulphur purple, facultative photoheterotrophic bacterium capable of growing phototrophically or chemotrophically as either a heterotroph or lithotroph in the presence or absence of O$_2$, depending on the energy source. This versatile lifestyle indicates its ability to seamlessly redirect its genetic potentials depending on the environmental conditions [1,2]. To accommodate these divergent lifestyles, *R. sphaeroides* has a number of inter-dependent regulatory mechanisms, the most important among them being the ability to delineate aerobic from anaerobic growth [3–5]. In this brief review, we will concentrate on some regulatory mechanisms found in *R. sphaeroides* cells undergoing a transition from aerobic to anaerobic growth, and the continuing role of these regulatory systems under anaerobic conditions.

Under anaerobic conditions in the light or dark, *R. sphaeroides* is capable of developing an extensive ICM (intracellular membrane) system, which arises as a series of differentiated invaginations from the cell membrane [6]. The ICM houses the photosynthetic apparatus.

Thus the response to O$_2$ is a major regulatory demarcation not only of growth states but also of morphology and ultrastructure. Under photosynthetic conditions, *R. sphaeroides* responds to the incident light intensity as well as the redox state of the external source of reductant by regulating the cellular abundance of the ICM, which can vary from 10 to 40% of cellular dry weight [6].

Oxygen control of ICM development has been shown to involve the two-component regulatory system PrrBCA, involving the membrane-localized histidine kinase PrrB, the response regulator PrrA and the membrane-anchored, Cu$^{2+}$-containing protein PrrC [7–10]. The default state of PrrB is in the kinase positive mode [11,12]. This fact alone suggests the existence of a mechanism preventing PrrB activation of PrrA in the presence of O$_2$. *R. sphaeroides* contains a highly branched aerobic electron-transport chain involving the bc$_1$ oxidoreductase, two intermediate cytochromes (soluble c$_2$ and membrane-bound c$_5$) and two terminal oxidase complexes (aa$_3$ and cbb$_3$) [13]. Under aerobic growth, reductant flow through the cbb$_3$ terminal oxidase results in the enhancement of the phosphatase activity of PrrB relative to its kinase activity, dephosphorylation of PrrA~P, causing PS (photosynthesis) gene expression to be ‘off’ in the presence of O$_2$ [11]. In the absence of O$_2$, the kinase activity of PrrB is increased relative to its phosphatase activity, causing PrrA~P to accumulate, turning on PS gene expression [11]. The unique role of the cbb$_3$ terminal oxidase is revealed in cbb$_3$ mutant strains where PS gene expression is turned on under conditions of high oxygen [14]. Conversely, the absence of the aa$_3$ terminal oxidase does not result in the aerobic expression of the PS genes [15]. A recent report reveals this direct interaction between the cbb$_3$ and PrrB [12].

Thus two crucial components of the regulatory system involving O$_2$ control of PS gene expression, as well as the photosynthetic lifestyle including CO$_2$ and N$_2$ fixation, are the Prr two-component system and the cbb$_3$ terminal oxidase. In the absence of PrrA, PS gene expression is virtually off [16]. These results demonstrate that the cbb$_3$ inhibitory signal requires the Prr system. Several independent lines of evidence reveal that cbb$_3$ is also involved in reductant flow under anaerobic conditions, and they are listed below: (i) expression of cbb$_3$ is greater under anaerobic than aerobic growth; (ii) extra copies of the ccoNOQP operon encoding the cbb$_3$ can repress PS gene expression under photosynthetic conditions; and (iii) a cco deletion can suppress an FmL mutant, which is normally unable to grow photosynthetically. Collectively, these results reveal or suggest an anaerobic function for the cbb$_3$ terminal oxidase. Yet, cells lacking a functional...
cbb\textsubscript{3} grow as well as the wild-type under photosynthetic conditions.

To learn more regarding the anaerobic role of the cbb\textsubscript{3}, we analysed the genome-wide expression of a cbb\textsubscript{3} mutant versus the wild-type using our \textit{R. sphaeroides} 2.4.1-specific gene chip. The use of the gene chip and its validation has been described in [1,2]. We see that, under these conditions, 232 genes/orfs (108 increased and 124 decreased) show significant, altered gene expression profiles in the cbb\textsubscript{3} mutant when compared with the wild-type. Many of these are present under more extensive examination. Nonetheless, we observed that a wide diversity of gene expression profiles involving the tricarboxylic acid cycle, cold-shock genes, cytochrome c peroxidase, glycine utilization and many more show significant changes in expression profiles in the cbb\textsubscript{3} mutant strain. Since cytochrome oxidase is known to be a respiratory enzyme for energy generation in aerobic organisms, its hidden function as a regulator of gene expression under both aerobic and anaerobic photosynthetic conditions has given it a new role and opened a new field, linking the cellular redox state and gene expression.

This conclusion raises the question: what are the source and the acceptor of this electron flow? It is quite probable that the oxidation of succinate is the source of this electron flow, distinguishing this flow from the cyclic photosynthetic electron flow resulting from the light reactions of PS. The acceptor for the anaerobic flow of reductant through the cbb\textsubscript{3} is somewhat more subtle and rests on several recent observations [17]. We have suggested that the anaerobic conversion of the penultimate carotenoid (Crt) SE (spheroidenone) into the end-product SO (spheroidenone) is generally prevented by the lack of a 2-oxo donor required for this conversion under anaerobic conditions [18]. This conversion is catalysed by the crtA gene product in the presence of O\textsubscript{2} [18]. We have shown that this 2-oxo group does not come from water under anaerobic conditions. We hypothesize that the 2-oxo donor is maintained in the reduced state by serving as the ultimate electron acceptor for reductant flow through the cbb\textsubscript{3} under anaerobic conditions [19], since, in cells growing photosynthetically, the ratio of SE to SO is approx. 10:1. When this flow is interrupted, e.g. as due to a mutant cbb\textsubscript{3} or the absence of the RdxB protein (a hypothetical iron-sulphur centre believed to interact with the cbb\textsubscript{3}), then the 2-oxo donor is not reduced and SO accumulates to high levels (SE/SO ratio 1:12) under photosynthetic conditions. The presence of the cco operon encoding the cbb\textsubscript{3} in trans in a cco deletion strain restores the correct ratio of SE to SO. In Figure 1, we show that the Crt coloration in various mutant strains affect the subunits of the cbb\textsubscript{3} complex. As can be seen, the coloration varies from bright pink (SO) to yellow (SE). Wild-type and the Q mutant are yellow, the P and O mutants are pink, and the N and cbb\textsubscript{3} deletions are in between. We believe that these results directly demonstrate the significance of electron flow through the cbb\textsubscript{3} to the source of the 2-oxo donor.

To address questions pertaining to the overall role of the Prr system in gene regulation, we performed several experiments involving the use of the gene chip with a PrrA mutant strain. Since a functional PrrA is required for PS growth [7], we had to conduct this experiment anaerobically in the dark using DMSO as the ultimate electron acceptor. Under these conditions, the PrrA mutant will grow anaerobically. In wild-type, under these same conditions, a functional ICM is formed, although cells are not growing photosynthetically in the dark. In the absence of PrrA, formation of the ICM is not possible [7]. Under aerobic conditions, PrrA is not required for growth [7].

Following the analysis of the gene chip experiment, we observed that approx. 20% of genes/orfs present in \textit{R. sphaeroides} underwent significant changes in expression profiles in the PrrA mutant relative to the wild-type; this amounts to more than 800 genes/orfs (J. Eraso and S. Kaplan, unpublished work). The affected genes/orfs mapped throughout the genome, i.e. on both chromosomes and all plasmids, with approx. 336 genes/orfs showing decreased expression levels and approx. 442 genes/orfs showing increased expression levels. Three general conclusions can be drawn from an examination of these results. Concerning the already known targets for PrrA and its homologues, and within the context of these particular experimental conditions, our results are consistent with previous data obtained in this laboratory as well as other laboratories. Secondly, the large number of genes found to be affected by PrrA, and an examination of their roles, uncovers new PrrA targets, some of which are under study, and concomitantly ascribes a more universal role to this response regulator, going beyond regulation of redox processes. For example, a large number of genes involved in general metabolic functions are regulated by PrrA. Likewise, the expression of genes involved in lipid metabolism is affected. In addition, a surprisingly large number of genes involved in translation are also regulated by PrrA. Clearly, the Prr system is firmly integrated into the overall cellular lifestyle of \textit{R. sphaeroides} either directly or indirectly. To address whether regulation by
PrrA is direct or indirect, an effort is being made to identify consensus binding sites for PrrA throughout the genome (L. Hao, C. Mackenzie, J.H. Roh, S. Kaplan and R. Haluk, unpublished work). Concerning indirect regulation by PrrA, a number of regulatory genes have been found to be regulated by PrrA, e.g. appA, tspO and ppaA, as well as some genes encoding other two-component systems, suggesting the existence of regulatory cascades. Finally, so far, only a positive regulatory role has been ascribed for PrrA and its homologues, but the present results indicate that negative regulation is also prevalent. Future characterization of target genes, as well as examination of consensus binding sites, will determine whether this negative regulation is direct or indirect.

It was indicated that the PrrA mutant cannot grow photosynthetically [7]. Figure 2, depicting the PS gene cluster (approx. 60 kb) in *R. sphaeroides*, contrasts the expression levels of PS genes in the wild-type and the PrrA mutant, for cells growing anaerobically in the dark on DMSO. These results clearly reveal the general shutdown of PS gene expression in the PrrA mutant. Genes involved in reaction centre, light-harvesting complexes, carotenoids and bacteriochlorophyll synthesis, namely *pfu*, *pubA*, *puc*, *crt* and *bch*, show a significant decrease in expression in the PrrA mutant,
Gene expression analysis of electron-transport chain genes of R. sphaeroides 2.4.1 in wild-type and PrrA mutant cells

Gene expression is given as mean signal value, derived from transcriptome analysis of wild-type and PrrA mutant cells. Wild-type expression values are represented by black bars and PrrA mutant values by white bars. Cells were grown anaerobically, sparged with 95% N₂ and 5% CO₂, in the dark, in the presence of DMSO as terminal electron acceptor. Transcriptome analysis was performed as indicated in [1,2].

Compared with the wild-type [44.6-fold for pufM (RSP0256), for example], whereas the genes encoding products necessary both under aerobic and anaerobic growth, such as cycA (RSP0296) and hemN (RSP0317) encoding cytochrome c₂ and coproporphyrinogen III oxidase respectively, are also regulated by PrrA, but not as significantly (3.5- and 2.3-fold respectively). In contrast, the urease cluster (ure) does not show any regulation by PrrA and neither does the regulatory gene ppsR (RSP0282). Similarly, in Figure 3, we contrast the expression profiles of those genes encoding components of the branched electron-transport chain in wild-type and the PrrA mutant. It is evident that a functional PrrA is essential for the expression of the bc₁ complex, cytochrome c₂ and the cbb₃ oxidase under anaerobic conditions. The former two are essential for photosynthetic electron transport, and the cbb₃ has been discussed above. The fbc genes, encoding the bc₁ complex, are positively regulated by PrrA, showing 2.8- to 4-fold increased expression when compared with the wild-type, whereas the genes constituting the cooNOQP operon, encoding the cbb₃ oxidase, show a similar 2.4- to 2.9-fold increase. Conversely, genes encoding the aa₃ terminal oxidase (cox) are either not affected by the absence of PrrA or only marginally affected. The aa₃ terminal oxidase is virtually non-existent under anaerobic growth, hence the very low levels of expression. However, cytochrome c₇, encoded by cycY (RSP0705), shows a 2.7-fold increase in expression in the mutant, compared with the wild-type, indicating that its expression is inhibited by a functional PrrA under anaerobic conditions.

Although we have only described selected elements of the regulation of PS gene expression with regard to O₂ control, we have amply demonstrated the importance of the cbb₃ branch of the aerobic electron-transport chain to the anaerobic lifestyle of R. sphaeroides and, together with the Prr system, we have demonstrated the existence of a deeply embedded global regulatory system in this organism. This regulatory system, whether directly or indirectly affecting the genes/orfs as revealed by the use of gene chip experiments, remains to be determined. However, it is evident that the cbb₃ regulon together with the Prr regulon points to the existence of a system of controls that appear to extend to almost all processes of the R. sphaeroides cell.

References

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