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Cell Signaling Through Redox Control of Gene Regulation in *Rhodobacter sphaeroides*[+]

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We reported earlier that inactivation of the *cbb*$_3$ oxidase leads to derepression of PS genes (PS^D) under aerobic conditions, which is accompanied by the oxygen-insensitive formation of the photosynthetic spectral complexes of *Rhodobacter sphaeroides*. Inactivation of the PrrBA two-component activation system in a *cbb*$_3$ background overrides the *cbb*$_3$– minus phenotype. The spectrum of PS genes which are derepressed under aerobic conditions by the inactivation of the *cbb*$_3$ oxidase (*puc*, *puf*, *hemA*, *bchE*, *hemN*, and *hemZ*) is coincident with those genes which are shown to be regulated by the PrrBA two-component system. Based on these and other findings, we proposed that the *cbb*$_3$ cytochrome *c* oxidase functions as an O_2/redox sensor which transduces an inhibitory signal to the membrane-bound sensor histidine kinase, PrrB, under aerobic conditions to prevent PS gene expression. The unique role of the *cbb*$_3$ oxidase in this sensory transduction pathway comes from studies of a *cbb*$_3$ lacking the CcoQ subunit. This mutant contains a fully functional *cbb*$_3$ oxidase but it produces spectral complexes aerobically like other Cco mutant strains, further suggesting that the *cbb*$_3$ oxidase itself is an O_2/redox sensor.

The finding that strong PS^D occurs in both BC1 and C2Cy mutants grown under high oxygen conditions allowed us to speculate that it is the interruption of electron flow into the *cbb*$_3$ oxidase which leads to aerobic PS^D. Employing myxothiazol it is clearly demonstrated that it is the extent of the electron flux through the *cbb*$_3$ oxidase, rather than the simple binding of an oxygen molecule *per se* to the *cbb*$_3$ oxidase, which determines the degree of PS^D. The greater the electron flow through the *cbb*$_3$ oxidase, the stronger the inhibitory signal generated by the *cbb*$_3$ oxidase which in turn represses the PrrBA two-component system, resulting in repression of PS gene expression.

As further proof that the signal transduction pathway originates within the structure of the *cbb*$_3$ oxidase, we demonstrate that altering the structure of the *cbb*$_3$ oxidase by changing a series of histidine residues involved in ligand coordination, to alanine all lead to the aerobic PS^D. However, only in the case of H407A were both the catalytic function and structure of the *cbb*$_3$ oxidase maintained. The H407A mutant contains near normal levels of apparently properly assembled *cbb*$_3$ oxidase containing normal amounts of heme *b*. Considering that the H407A mutant contains more *cbb*$_3$ oxidase activity than the wild type control, which contains a single copy of the *ccoNOQP* operon, oxygen-insensitive formation of spectral complexes in this mutant implies that either the His^{407} itself or precise placement of the low spin heme might be required in order for the *cbb*$_3$ oxidase to generate the inhibitory signal to repress PS gene expresion in the presence of O_2. The removal of His^{407}, as well as the non-polar deletion of the *ccoQ* gene, unambiguously dissociates the sensory function of the *cbb*$_3$ oxidase from its catalytic function, reinforcing the idea that the CcoQ subunit of the *cbb*$_3$ oxidase and the His^{407} residue or the low spin heme are intimately involved in signal generation and transduction.

The *cbb*$_3$ oxidase has excellent properties as an oxygen sensor in order to provide for the orderly control of PS gene expression in *R. sphaeroides*: i) it has a high affinity for O_2 (*Km* of *B. japonicum cbb*$_3$ oxidase is 7 nM which is 6- to 8-fold lower than that for the *aa*$_3$ oxidase): and ii) it is present in cells grown under anaerobic conditions. The high affinity of the *cbb*$_3$ oxidase for O_2 enables *R. sphaeroides* not to activate the PrrBA two-component system until O_2 tensions in the environment fall to sufficiently low levels. The anaerobic presence of the *cbb*$_3$ oxidase allows *R. sphaeroides* both to maintain transcriptional control of the PS genes under anaerobic conditions as well as to quickly turn off the PrrBA system as soon as it is exposed to O_2.

How then does the inhibitory signal move from the *cbb*$_3$ oxidase to the membrane-bound PrrB histidine kinase? We suggest that the CcoQ subunit, which is a part of the *cbb*$_3$ complex, can monitor the "volume" of electron flow through the *cbb*$_3$ oxidase. Further, recent genetic studies proposed that the membrane-bound PrrC protein, whose gene is located immediately upstream of *prrA*, is located between the *cbb*$_3$ oxidase and the PrrBA two-component system in the signal transduction pathway.

R. sphaeroides contains two functional cytochromes *c* under normal physiological conditions, which participate in electron transfer between the membrane-associated *bc*$_1$ complex and the two terminal cytochrome *c* oxidases. The soluble periplasmic cytochrome c_2 supports both respiratory and photosynthetic ETC, while the membrane-anchored cytochrome c_y participates in only respiratory electron transfer. Based upon our assumption that the "extent" of electron flow through the *cbb*$_3$ oxidase ultimately determines the level of PS gene expression under aerobic conditions, then the differential expression of both the *puc* and *puf* operons observed in the C2 and Cy mutants grown under 30% O_2 conditions permits us to assign, albeit indirectly, the relative contribution of cytochromes c_2 and c_y to channel electrons from the *bc*$_1$ complex to the *cbb*$_3$ oxidase. From these results, we suggest that under 30% O_2 conditions the membrane-bound cytochrome c_y is approximately 2-3 times more effective in its ability to channel electrons from the *bc*$_1$ complex to the *cbb*$_3$ oxidase than is cytochrome c_2.

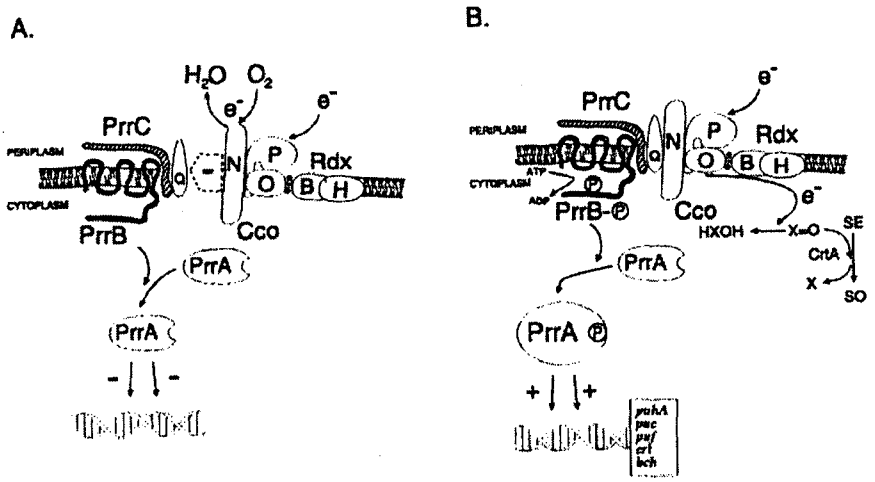
The *puf* operon, which appears to be under the exclusive control of the PrrBA system, was shown to be derepressed to similar levels in the BC1, C2Cy, CCONΔ, and CCOQΔ mutant strains. However, in the case of the *puc* operon its degree of derepression in the aforementioned mutants was highly variable, i.e., derepression of the *puc* operon in the BC1 and C2Cy was substantially higher than that observed for the CCONΔ and CCOQΔ mutant strains. These differences in *puc* and *puf* operon expression imply that "factors", in addition to the PrrBA activation system, are operative together with the PrrBA system in regulating the *puc* operon. These factors, in a *cbb*$_3$-PrrBA-independent manner, sense another redox signal associated with the interruption of electron flow through the *bc*$_1$ complex to enhance *puc* operon expression. We postulate that the redox state of the quinone pool, which is positioned upstream of the *bc*$_1$ complex in the ETC, might be the source of another signal which is transduced to enhance *puc* operon expression independently of the *cbb*$_3$-PrrBA signal transduction pathway. The redox state of the quinone pool can be affected by the rate at which electrons are removed to the terminal oxidases under aerobic conditions or to the terminal reductases such as DMSO reductase under anaerobic conditions, when a steady flow of electrons from a given organic electron donor is entering the pool. In addition, changes in the light intensity also affect the redox state of the quinone pool. Therefore, under 30% O_2,

inactivation of the bc_1 complex or both cytochromes c_2 and c_y is likely to shift the quinone pool toward a more reduced state, which might preferentially enhance the derepression of the *puc* operon relative to the *puf* operon.

What then might sense the redox state of the quinone pool? The best candidate to our knowledge is the AppA-antirepressor-PpsR repressor system.

On the basis of the results presented here, as well as those reported earlier, we present a model describing the interrelationship between the regulation of the PS genes and the activity of the ETC in *R. sphaeroides*. This model together with other recent studies of the anaerobic regulator FnrL, which participates in the selective control of both *puc* and the tetrapyrrole biosynthesis genes such as *hemA*, *hemN*, *hemZ*, and *bchE* as well as the *ccoNOQP* operon, as conditions become increasingly less aerobic, allows us to fully describe the regulation of PS gene expression *in R. sphaeroides*.

In summary, we demonstrate that the regulation of PS gene expression in *R. sphaeroides* is closely coupled with the activity of the ETC, i.e., the functional state of the cbb_3 oxidase and the redox state of the quinone pool. The fact that the cbb_3 oxidase has the dual function as both a terminal oxidase and O_2 sensor and it, together with the PrrBA two-component system, constitutes a signal transduction pathway provides a new paradigm for O_2 sensing and gene regulation. The advantage of redox sensing through the ETC, as demonstrated here, appears to be the ability to respond rapidly and precisely to environmental stimuli as well as to provide for a mechanism to integrate all cellular metabolic activities.



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