# 초 청 강 연 초 록

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## The role of the *cbb*$_3$ cytochrome *c* oxidase as an oxygen sensor in the photosynthetic bacterium, *Rhodobacter sphaeroides*

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The photosynthetic bacterium, *Rhodobacter sphaeroides*, can perform photosynthesis only under oxygen-limiting and anaerobic conditions. When oxygen tensions fall below ~3%, intracytoplasmic membrane containing the photosynthetic apparatus is formed as a result of invaginations of the cytoplasmic membrane. The photosynthetic apparatus is comprised of two light-harvesting complexes (B875 and B800-850) and a photochemical reaction center which forms the photosynthetic electron transport chain (ETC) together with the *bc*1 complex as well as the mobile electron carriers such as quinone pool and cytochrome $c_2$. The photosynthesis (PS) genes, which encode the apoproteins of the photosynthetic apparatus and proteins involved in biosynthesis of photopigments (bacteriochlorophyll and carotenoid), are induced in response to the reduction in oxygen tensions. Therefore, the primary signal leading to the turn-on or off of PS gene expression in *R. sphaeroides* is oxygen.

The PrrBA two-component system is one of the major regulatory pathways which participate in the regulation of PS genes in response to changes in oxygen tensions. The PrrBA two-component system is a typical bacterial two-component system consisting of the membrane-localized PrrB sensory

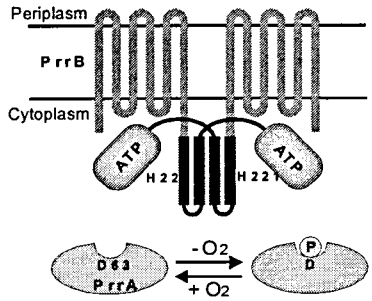


Fig. 1. The PrrBA two-component system. H221, histidine-221 in the DHp domain which is autophosphorylated; ATP, CA domain; D63, aspartate-63 in the receiver domain of the response regulator PrrA which receives the phosphate group from PrrB. The phosphorylated PrrA is an active form which serves as the transcriptional activator for the induction of PS genes under anaerobic conditions.

histidine kinase and its cognate PrrA response regulator (Fig. 1). PrrB consists of two structurally and functionally distinct domains. The C-terminal domain (amino acids 183 to 462) of PrrB contains a CA (Catalytic ATP-binding) domain and a DHp (Dimerization and Histidine phosphotransfer) domain which are directly involved in phosphorylation and dephosphorylation of the cognate response regulator PrrA . The N-terminal membrane-spanning domain contains six transmembrane helices framing three periplasmic and two cytoplasmic loops and has been suggested to fuction as a sensing domain. Recently it was demonstrated that the central portion of the PrrB transmembrane domain including the second periplasmic loop and flanking transmembrane helices 3 and 4 might be the essential segment of the transmembrane-spanning domain engaged in the sensing function of PrrB. The chemistry of the kinase reaction involves two half reactions: i) autophosphorylation of the conserved histidine (His-221 in *R. sphaeroides* PrrB) in which the g-phosphoryl group of ATP is transferred to the side chain of the histidine residue, ii) the phosphotransfer

reaction in which the phosphoryl group on the histidine is moved to an aspartate residue (Asp-63 in PrrA) present in the receiver domain of the response regulator. In addition, it was demonstrated that PrrB possesses a phosphatase activity to hydrolyze the phosphoryl group from phosphrylated PrrA. The kinase-dominant state of PrrB is favored in low-oxygen or anaerobic conditions, resulting in the induction of PS gene expression, whereas the relative propensity for the phosphatase-dominant state increases with increasing oxygen tensions in the environment. When the sensing domain (membrane domain) of PrrB is altered by mutagenesis, the altered PrrB is in the kinase-dominant mode even under high oxygen conditions. The overexpression of the *prrB* gene in *R. sphaeroides* leads to spectral complex formation under highly aerobic conditions. These observations were interpreted to suggest that the intrinsic (default) state of the relative PrrB activity is in the kinase-dominant mode and that a signal controlling the ratio of kinase/phosphatase exists under high-oxygen conditions.

Since the PrrB histidine kinase does not appear to contain any sequence motif for any known redox prosthetic groups like heme, flavin, and iron-sulfur center, it was unclear how it senses and responds to changes in the $O_2$ tension in order to regulate the phosphorylation state of the cognate response regulator PrrA. The first clue that the $cbb_3$ oxidase encoded by the *ccoNOQP* operon, was related to the PrrBA two-component system was obtained following the observation that inactivation of the $cbb_3$ oxidase led to the formation of the photosynthetic apparatus in *R. sphaeroides*, even under highly aerobic conditions. The $O_2$-insensitive formation of spectral complexes in Cco null mutants is the result of the derepression of PS genes under aerobic conditions. The spectrum of PS genes that are derepressed under aerobic conditions by the inactivation of the $cbb_3$ oxidase is coincident with those genes which are shown to be regulated by the PrrBA two-component system. Inactivation of the PrrBA system in a Cco

null mutant abolishes the Cco-minus phenotype. Both findings enabled us to suggest that the PrrBA two-component system resides downstream of the $cbb_3$ oxidase in the signal transduction pathway, and that the $cbb_3$ oxidase normally generates a signal under aerobic conditions which shifts the relative equilibrium of PrrB activity from the kinase mode to the phosphatase-dominant mode, resulting in the repression of PS gene expression

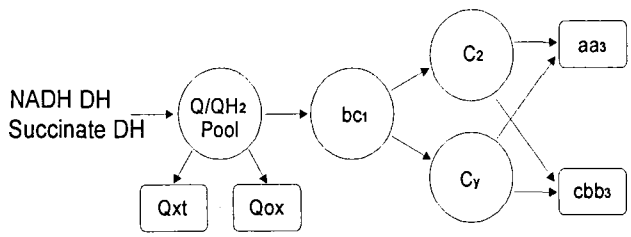


Fig. 2. Aerobic respiratory electron transport chain of *R. sphaeroides*. Abbreviations: DH, dehydrogenase; $Q/QH_2$, ubiquinone/ubiquinol; Qxt and Qox, Qxt- and Qox quinol oxidases.

Inactivation of the $aa_3$ oxidase, which shares the upstream ETC components with the $cbb_3$ oxidase, led to no change in PS gene expression (*puc* and *puf*) under highly aerobic conditions. Since the $aa_3$ cytochrome *c* oxidase is the major cytochrome *c* oxidase under highly aerobic conditions, where it is responsible for ~65-70% of the cytochrome *c* oxidase activity, the possibility is ruled out that the aerobic derepression of the PS genes observed in Cco null mutants is the result of either changes in the redox state of electron carriers upstream of the $cbb_3$ oxidase or a decrease in the proton motive force across the membrane.

Inactivation of either the $bc_1$ complex or both cytochromes $c_2$ and $c_y$, through which electrons are transferred from the quinone pool to both cytochrome *c* oxidases, also brings about derepression of PS genes under aerobic conditions, as observed for the Cco mutants. This finding indicates that it is the interruption

of electron flow into the *cbb*$_3$ oxidase that leads to the aerobic derepression of the PS genes. Subsequently, it was demonstrated that it is the rate or volume of electron flow through the *cbb*$_3$ oxidase, rather than the binding per se of an $O_2$ molecule to the *cbb*$_3$ oxidase that ultimately determines the extent of PS gene regulation by the PrrBA two-component system. The greater the volume of electron flow to the *cbb*$_3$ oxidase, the stronger the signal generated by the oxidase to shift the equilibrium of PrrB activity toward the phosphatase mode, resulting in the repression of PS genes. Therefore, under aerobic conditions where $O_2$, the substrate of the *cbb*3 oxidase, is sufficient for turnover of the oxidase, PS gene expression is repressed.

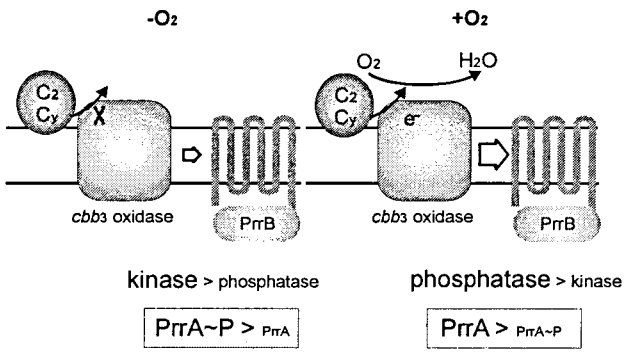


Fig. 3. Model for $O_2$ signaling through the *cbb*$_3$-PrrBA signal transduction pathway. $O_2$, anaerobic conditions; $+O_2$, aerobic conditions; $C_2$ and Cy, cytochromes *c*$_2$ amd *cy*. The size of the arrows indicates the strength of the inhibitory signal emanating from the *cbb*$_3$ cytochrome *c* oxidase.

The model for $O_2$ sensing through the *cbb*$_3$-PrrBA signal transduction pathway was suggested by Oh and Kaplan (2001). The intrinsic (default) state of the PrrB histidine kinase is in the kinase-dominant mode. That is, when there is no external signal to the PrrB histidine kinase, the equilibrium for the relative PrrB kinase/phosphatase activity is in favor of kinase activity. The *cbb*$_3$ cytochrome *c*

oxidase acts as an $O_2$ sensor. The extent of electron flow through the *cbb*$_3$ oxidase, which is dependent on $O_2$ availability, determines the relative activity of PrrB: the greater the electron flow through the *cbb*$_3$ oxidase (the higher the $O_2$ tension), the stronger the inhibitory signal generated by the *cbb*3 oxidase, which shifts the PrrB activity away from the kinase mode toward the phosphatase mode. When $O_2$ tensions are reduced or under anaerobic conditions, the reduced or interrupted electron flow through the *cbb*$_3$ oxidase alleviates or abolishes the inhibitory signal and PrrB returns to its default state, i.e., the kinase-dominant mode, to induce PS gene expression.