In vitro studies of sensor kinase and response regulator proteins of the Prr two-component signal transduction pathway in *Rhodobacter sphaeroides*

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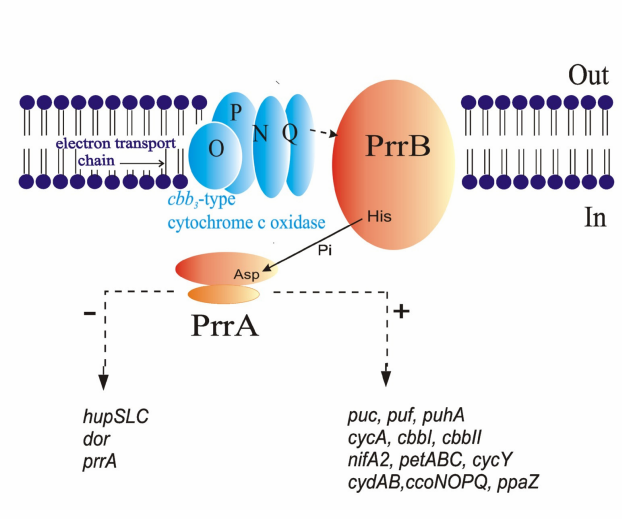

Fig. 1. The Prr two-component signal transduction pathway in Rhodobacter sphaeroides

Prr is a global regulatory system belonging to the 'two-component' family of signal transduction proteins (Fig. 1). It controls a large and diverse range of genes in *Rhodobacter sphaeroides* including those for synthesis of the photosynthetic apparatus, electron transport, nitrogen and carbon fixation, anaerobic respiration, [NiFe] hydrogenase and aerotaxis, in response to changing conditions of environmental redox potential; Prr is therefore a pivotal regulator in the complex switch between aerobic and anaerobic lifestyles and the optimum use of reducing power in this versatile bacterium. The aim of our work is to characterise fully the mechanisms of signal sensing, signal transduction and DNA target binding, in order to provide insights into how globally-important processes such as carbon and nitrogen fixation, as well as photosynthesis, are coodinately regulated by just one system in one bacterium. PrrB is the membrane-bound sensor kinase that senses changes in redox potential. Upon anaerobiosis, PrrB becomes autophosphorylated and transfers the phosphoryl signal to response regulator PrrA. Once phosphorylated, PrrA~P then positively or negatively regulates gene expression.

Recent advances: PrrB.

Previously we showed that the purified, detergent-solubilised intact membrane sensor PrrB is functional in autophosphorylation, phosphotransfer and phosphatase activities. Here, we confirm that it also senses and responds directly to its environmental signal, redox potential, *in vitro*; strong autophosphorylation of PrrB occurred in response to dithiothreitol-induced reducing conditions (and levels increased in response to a wide 0.1 - 100 mM DTT range), whilst under oxidising conditions, PrrB exhibited low, just detectable levels of autophosphorylation (Fig. 2). The clear response of PrrB to changes in reducing conditions confirmed its suitability for *in vitro* studies to identify modulators of its phosphorylation signalling state, and so far has

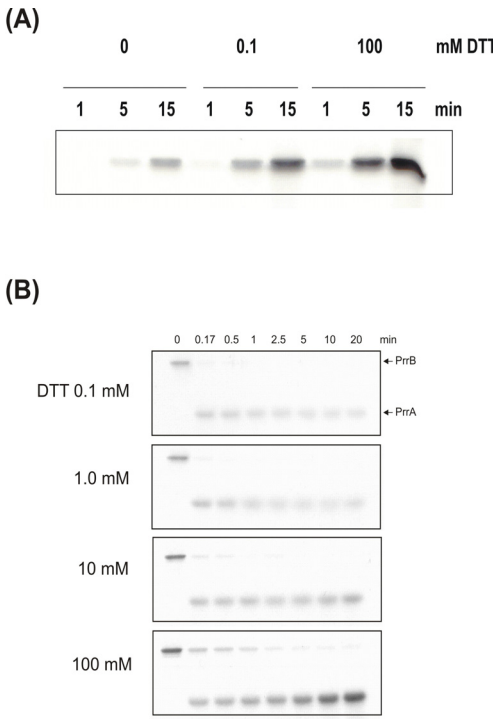

Fig. 2. Modulation of PrrB autophosphorylation and phosphotransfer to PrrA by dithiothreitol in vitro. (A) Effect of DTT on PrrB autophosphorylation in assays initiated using[γ-32P]ATP. (B) Effect of DTT on PrrB autophosphorylation and subsequent phosphotransfer to PrrA. PrrB was allowed to autophosphorylate in the presence of DTT for 20 min; PrrA was then added (final PrrB:PrrA ratio of 1:2.5), and levels of PrrB~P and PrrA~P measured.

been used to investigate whether PrrB might sense more than one redox-related signal, such as signals of cell energy status. NADH, ATP and AMP were found to exert no detectable effect on maintenance of the PrrB~P signalling state. By contrast, ADP produced a very strong increase in PrrB~P dephosphorylation rate, presumably through the back-conversion of PrrB~P to PrrB.

Recent advances: PrrA.
Using gel filtration, analytical centrifugation and NMR diffusion measurements, our recent studies of PrrA have shown that treatment of the protein with a phosphate analogue, BeF_3^-, results in dimerisation of the protein, producing a protein that binds DNA. Upon addition of BeF_3^-, the inhibitory activity of the N-terminal regulatory domain on the C-terminal DNA-binding domain is relieved, after which PrrA becomes capable of binding DNA as a dimer. The interaction surface of the DNA-binding domain with the regulatory domain of PrrA was identified by NMR as being a well-conserved region centred on helix α6, which is on the face opposite from the DNA recognition helix. This suggests that there is no direct blockage of DNA binding in the inactive state, but rather that PrrA dimerisation promotes a correct arrangement of two adjacent DNA-binding domains that recognises specific DNA binding sequences.

Publications
Potter, C.A., Jeong, E-L., Williamson, M.P., Henderson, P.J.F. & Phillips-Jones, M.K. (2006) Redox-responsive *in vitro* modulation of the signalling state of the isolated PrrB sensor kinase of *Rhodobacter sphaeroides* NCIB 8253. *FEBS Lett*. **580**, 3206-3210.

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