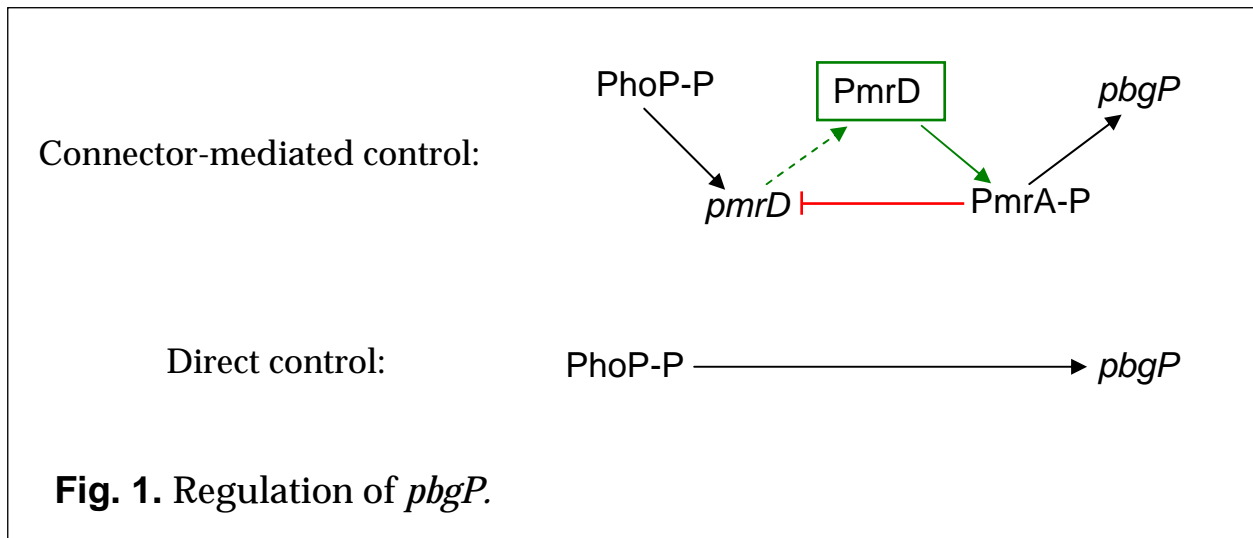


## Dynamical properties of connector-mediated pathways in two-component signal transduction

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Two-component signal transduction systems are the primary molecular mechanisms allowing bacterial cells to sense and respond to changes in the surrounding environment. Currently, hundreds of such systems are known. The main components of a prototypic two-component system are two proteins, a sensor kinase and a response regulator. The sensor kinase has the ability to phosphorylate (and/or dephosphorylate) the response regulator, depending on the strength of the environmental signal. The response regulator has the ability to regulate gene transcription, and phosphorylation enhances this ability.

While a single two-component system usually responds to one or just a few different signals, in real life bacteria need to integrate multiple signals acting simultaneously. To this end, bacteria have evolved interactions between different two-component systems. Such interactions allow one system to react to signals sensed by another system. While this general property is possessed by regulatory systems with different architectures, the functional and dynamical features of the interconnected two-component systems depend on the type of connection used. We investigated the dynamics of an emerging design that relies on a small protein connecting two two-component systems at the post-translational level. In *Salmonella enterica*, the *pbgP* gene, responsible for resistance

to the antibiotic polymyxin B, is controlled by the PhoP/PhoQ and PmrA/PmrB two-component systems. We focused on the control by PhoP/PhoQ, which is carried out in an indirect manner via the connector protein PmrD, which stabilizes the phosphorylated form of PmrA, allowing the latter to activate *pbgP* transcription (Fig. 1). We have compared the dynamics of this control circuit with the case when *pbgP* is directly controlled by PhoP (this architecture was reconstituted *in vivo* in a *Salmonella* strain). The inputs for the two circuits were the concentrations of  $Mg^{2+}$ , which is the signal sensed by PhoP/PhoQ; the system is activated in low  $Mg^{2+}$  and repressed in high  $Mg^{2+}$ . The output was the level of *pbgP* mRNA as measured in quantitative real-time PCR experiments.

In our experiments, we observed that the connector-mediated pathway demonstrated activation and inactivation delays compared to the direct regulation pathway. Deactivation delays were especially pronounced. We also compared the steady-state induction ratios for the two architectures. (An induction ratio is the ratio of the output level under inducing conditions to that under repressing conditions.) We found that the induction ratios for the connector mediated pathway were substantially higher than those of the direct regulation pathway, and designated this effect “signal amplification”.

We developed ordinary differential equation models for the connector-mediated and direct pathways. Analytical investigation of the steady-state equations showed that, under natural assumptions verified by model fitting to experimental data, signal amplification is a direct consequence of the connector-mediated architecture. By performing computational experiments with randomized parameters, we showed that activation/deactivation delays are typical for the connector-mediated pathways.