Modeling raises the possibility that BvgAS phosphorelay kinase activity may be constitutive

Sotiria Lampoudi[1], Robin R. Hulbert[2], Linda R. Petzold[1], Peggy A. Cotter[2]

***Short Abstract* — A key question in the study of two-component signal transduction systems is: which of their activities are constitutive and which are regulated by the signal? This has been definitively answered for only a handful of systems. In the case of the BvgAS phosphorelay, an unorthodox two-component system which regulates virulence in *Bordetella sp.*, the answer has been tacitly assumed to be that the kinase activity of BvgS (autophosphorylation) is the signal-modulated step. We present experimental and modeling results which cast doubt on that assumption, and suggest that modulation of receiver activities (hydrolysis and relay) is also sufficient for phenotypic phase regulation.**

***Keywords* — Mathematical modeling, two-component systems, signal transduction, phosphorelay.**

I. Introduction

TWO-component signal transduction systems typically comprise a sensor kinase protein, often membrane-bound, and a response regulator protein. In orthodox bacterial two-component systems the sensor kinase becomes phosphorylated, at the expense of ATP, on a conserved *His* residue in response to a signal, and then transphosphorylates the response regulator on a conserved *Asp* residue. The activity of the response regulator, often DNA binding, is modulated by its phosphorylation state.

The BvgAS phosphorelay is an unorthodox two-component system which contains two additional domains. After the BvgS kinase autophosphorylates on a *His*, it relays the phosphoryl group first to a conserved *Asp*, then to another conserved *His*, before finally transphosphorylating the response regulator BvgA on an *Asp*. BvgS also functions as a phosphatase of its cognate response regulator by reverse phosphotranfer, followed by relay to the intermediate *Asp*, from which the phosphoryl group can be lost by hydrolysis [1]. Ultimately, the transcriptional profile of the cell depends on the level of phosphorylated response regulator. The phosphorelay is the mechanism regulating that level in response to the level of signal.

For most phosphorelays, as well as many orthodox two-component systems, it is not known how the interaction between the stimulus and the sensor kinase modulates the flow of phosphate through the relay. It has been shown that the signal (AI-1) directly down-regulates the kinase activity of LuxN in *Vibrio harveyi* [2], while the issue of whether the kinase or phosphatase activity is regulated in EnvZ remains controversial [3,4]. Modeling of the novel P1027S-BvgS mutant suggests that, in the case of the BvgAS phosphorelay, regulation of either the kinase activity (autophosphorylation) or one of the receiver activities (hydrolysis or relay) is sufficient to achieve the phenotypic phase switching for which BvgAS is responsible [5].

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[1]Department of Computer Science, UC Santa Barbara. E-mail: slampoud@cs.ucsb.edu, petzold@cs.ucsb.edu

[2]Department of Molecular, Cellular and Developmental Biology, UC Santa Barbara. E-mail: hulbert@lifesci.ucsb.edu, cotter@lifesci.ucsb.edu

II. Method

We built a 10 state, 7 parameter ODE model of the BvgAS phosphorelay upstream of DNA binding. We used wild type and known mutant data in a two-step optimization routine to fit the kinetic parameters. We then considered three scenarios for the potential effect of a novel BvgS mutation (P1027S): a) that it increases autophosphorylation, b) that it decreases hydrolysis from the receiver, and c) that it increases relay from the receiver. We eliminated hypothesis (a) on the basis of mismatch between the model and data. The data was insufficient to discriminate between (b) and (c).

III. Conclusion

The elimination of the hypothesis that the P1027S mutation directly affects the kinase activity of BvgS, taken together with the phenotype of P1027S-BvgS, suggests that direct modulation of *receiver* activities by signal is sufficient for phenotypic phase switching by BvgS.

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