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UNRAVELING THE BVGAS PHOSPHORELAY

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Bacteria sense and respond to environmental stimuli using pairs of proteins called two-component systems. These are composed of a histidine kinase sensor protein, which autophosphorylates in the presense of the signal being sensed, and a response regulator protein, which is typically involved in binding DNA and controlling gene transcription. The information that a signal is being sensed is relayed from the sensor to the response regulator via a phosphotransfer step. A more sophisticated variant of the two-component system, the phosphorelay, contains two additional signaling domains and two additional phosphotransfer steps.

The BvgAS phosphorelay controls virulence in the *Bordetella* family of respiratory pathogens. *Bordetella pertussis* is the strictly human-adapted etiological agent of whooping cough, and causes acute infections. *Bordetella bronchiseptica* causes chronic respiratory infections in a variety of four-legged mammals. BvgAS employs a four step His-Asp-His-Asp phosphorelay from the sensor protein BvgS to the response regulator BvgA.

We have developed a family of computational models and simulations of the BvgAS signal transduction and gene expression pathway, which we use to explore both quantitative and qualitative questions. The ultimate goal is to unravel how the phosphorelay works and what are its advantages over the more simple two-component systems.

The computational models are being developed in a methodical fashion. In vitro experiments are modeled by ordinary differential equations of chemical kinetics. The ODE models are used in optimization in order to obtain kinetic parameter estimates. This suggests gaps in the experimental literature and experiments best suited to fill those gaps, which are then performed.

Bordetellae display at least three distinct phenotypic phases, each characterized by maximal expression of some genes and minimal expression of others. It is postulated that each phase corresponds to the bacteria's attempts to thrive in different conditions. Thus, *minus* phase would be associated with survival outside a host, in a nutrient-deprived environment, *plus* phase would be associated with infection, and *intermediate* phase would be associated with (aerosol) transmission [1]. BvgAS may mediate this by functioning more like a rheostat than a switch. By fitting a model of a typical two-component system to *in vitro* BvgAS data, we eliminated the hypothesis that a two-step phosphotransfer can produce anything other than switch-like behavior.

Our latest efforts have been focused on determining the biochemical mechanism behind differences observed in *in vitro* phosphorylation assays using purified BvgS and BvgA from *B. pertussis* vs *B. bronchiseptica*. The assays show differential levels of BvgA and

BvgS phosphorylation between these two *Bordetella* species, both in wild type and mutant comparisons. We currently have two competing theories regarding what biochemistry may account for the observed differences. According to one theory, it is the rate of autophosphorylation of BvgS that differs between the two species. According to the other, the BvgS of the two species hydrolyzes phosphoryl groups at different rates, making the transmission of signal in one species less efficient than in the other. It is important to distinguish between the two theories in order to establish whether the intermediate phenotypic phase -- the postulated aerosol transmission phase-- is the result of intermediate levels of signal sensing or intermediate levels of signal propagation in the transduction network.

A model of the phosphorelay, containing all the biochemical reactions upstream of DNA binding (autophosphorylation, two relay steps, hydrolysis from the receiver domain, and transphosphorylation of the response regulator) was developed. We implemented each of the two competing theories as a variant of the model, resulting in a family of two models. We then performed computational simulations, which exactly mimicked the *in vitro* assays, and compared the outcome to the *in vitro* experimental data. This indicated that both theories matched some, but not all, of the experimental data. In order to optimally discriminate between the two theories, we proposed a series of further *in vitro* assays, based on measurements of inorganic phosphate. These experiments are currently being carried out.

Although both two-component regulatory systems and phosphorelays are involved in the control of virulence gene expression in many bacterial pathogens, including several highly virulent species, phosphorelays tend to sit at the top of large regulatory hierarchies and thus serve as master regulators. Inactivation of such systems frequently results in bacteria that are completely avirulent. Phosphorelays therefore represent attractive therapeutic targets. A thorough understanding of how they function should lead to the development of efficacious antimicrobial strategies, compounds and vaccines.

[1] P. A. Cotter and A. M. Jones, “Phosphorelay control of virulence gene expression in Bordetella”, Trends Microbiol. 11: 367-73, 2003.