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Positive feedback and temperature mediated molecular switch controls differential gene regulation in *Bordetella pertussis*

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ABSTRACT

Based on the phosphorelay kinetics operative within BvgAS two component system we propose a mathematical framework for signal transduction and gene regulation of phenotypic phases in *Bordetella pertussis*. The proposed model identifies a novel mechanism of transcriptional interference between two promoters present in the *bvg* locus. To understand the system behavior under elevated temperature, the developed model has been studied in two different ways. First, a quasi-steady state analysis has been carried out for the two component system, comprising of sensor BvgS and response regulator BvgA. The quasi-steady state analysis reveals temperature induced sharp molecular switch, leading to amplification in the output of BvgA. Accumulation of a large pool of BvgA thus results into differential regulation of the downstream genes, including the gene encoding toxin. Numerical integration of the full network kinetics is then carried out to explore time dependent behavior of different system components, that qualitatively capture the essential features of experimental results performed *in vivo*. Furthermore, the developed model has been utilized to study mutants that are impaired in their ability to phosphorylate the transcription factor, BvgA, of the signaling network.

1. Introduction

One of the important functional aspects of living organisms is to respond to the sudden changes made in their environment, and to make appropriate changes in the cellular or subcellular level for survival. Direct manifestations of such changes at the subcellular level are the expression/repression of single or multiple genes controlling different functional behavior of an organism (Alon, 2007). To achieve this, living system utilizes concerted biochemical network composed of several feedback mechanism (Tyson et al., 2003; Tyson and Novák, 2010). The human pathogen *Bordetella pertussis*, a gram negative bacteria and causative agent for the disease whooping cough (Preston et al., 2004), is no exception to the aforesaid behavior. At 25 °C, while freely moving in the environment their pathogenic properties remain dormant. But, when they are within the host at 37 °C, their virulent properties come into play. In the laboratory the reverse effect, i.e., suppression of pathogenic behavior is observed using $MgSO_4$ or nicotinic acid (Beier and Gross, 2008; Cotter and Jones, 2003). The virulent behavior of *B. pertussis* within host, in response to sudden environmental change, has been experimentally studied and has been found to be operative through BvgAS two component system (TCS) (Beier and Gross, 2008; Cotter and Jones, 2003). The TCS comprises of transmembrane sensor BvgS and response regulator BvgA where signal flows through this pair via a four step (His-Asp-His-Asp) phosphorelay mechanism.

As a response to temperature elevation in the environment, the response regulator BvgA becomes active (the phosphorylated dimer) within each bacterium, which in turn exerts a positive feedback on its own operon, the *bvg* operon. Positive feedback loop thus increases the active form of BvgA in a switch like manner. In other words, once the BvgAS two-component machinery becomes operative, large pool of active BvgA either repress and/or express several downstream genes where the phosphorylated dimer of BvgA plays the leading role by acting as transcription factor (TF) (Steffen et al., 1996). In the laboratory condition at 37 °C and in the absence of $MgSO_4$ or nicotinic acid BvgA activates transcription of virulence activated genes (*vag*), as well as represses transcription of virulence repressed genes (*vrg*). Due to this reason *bvg* locus was earlier called *vir* due to its connection to virulence (Beier and Gross, 2008; Cotter and Jones, 2003; Weiss and Falkow, 1984). In *B. pertussis*, *vrg* loci encodes outer membrane whereas in *B. bronchiseptica*, *vrg* controlled genes encode motility and survival from nutrient limitation condition. In *Bordetella* spp., *vag* loci encodes genes responsible for adherence, toxins (including pertussis toxin in *B. pertussis*, a type III secretion system and BvgAS itself (due to autoregulation).

Based on the binding affinity of TF to the respective promoters, different types of downstream genes are regulated in *Bordetella* spp. and have been broadly grouped into four classes, e.g., class 1, class 2, class 3 and class 4 (Beier and Gross, 2008; Cotter and

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Jones, 2003). Class 1 genes encompass genes that are responsible for encoding toxins, such as adenylate cyclase (*cyaA-E*) and pertussis toxin (*ptxA-E*). Class 2 genes express proteins responsible for adherence, such as *fhaB* that encodes filamentous hemagglutinin. Among all the four classes of genes, class 3 genes show a unique behavior, although its functional activity is not known till date (Beier and Gross, 2008; Cotter and Jones, 2003). The only well characterized class 3 gene found in *B. pertussis* is known as *bipA*. The final one, class 4 genes have been reported to encode *frlAB* in *B. bronchiseptica* and is responsible for motility. It is important to mention that expressions of class 3 and class 4 gene are not observed in *B. pertussis* under the influence of temperature elevation. To be specific, class 3 gene expression has been observed in *B. pertussis* only under the influence of intermediate concentration of $MgSO_4$ and class 4 gene under low concentration of $MgSO_4$ in *B. bronchiseptica* (Beier and Gross, 2008; Cotter and Jones, 2003). In terms of *vir* regulated genes class 4 gene thus belongs to *vrg* whereas class 1 and class 2 genes belong to *vag*. Expression and/or repression of the four classes of downstream genes is controlled by strong and/or weak binding sites (for TF) present in the promoter region of the respective genes. Among these, promoter region of class 4 gene has the strongest affinity for TF. Promoter region of class 2 and class 3 genes have medium affinity for TF, whereas promoter region of class 1 gene has the weakest affinity for TF. On the basis of the promoter regions' affinity for TF it is thus expected that expression and/or repression of four classes of downstream genes in *Bordetella* spp. would show a differential pattern in their temporal dynamics.

Keeping these aforesaid phenomenological information in mind we have developed a mathematical model based on biochemical interactions taking place within *B. pertussis* under the influence of temperature elevation. The objective of present work is twofold. First, we aim to understand the molecular switch operative in BvgAS TCS and to identify the key players responsible for amplification of TF. Second, through our model we aim to regenerate qualitative features of the network and to mimic different phenotypic states of *B. pertussis* under temperature elevation, as well as their expression level due to different mutation.

2. The model

To understand the mechanism for temperature induced activation of *bvg* locus and differential regulation of the downstream genes, we propose a kinetic model in the following.

2.1. The bvg locus

Experimental studies in *B. pertussis* suggest multi-promoter activities in *bvg* operon (Roy et al., 1990; Scarlato et al., 1990, 1991). Out of the four promoters, P_{AS1}, P_{AS2}, P_{AS3} and P_{AS4}, present in the *bvg* locus (see Fig. 1), only P_{AS2} is known to be constitutively active under non-inducing condition (25 °C) and is *bvg* independent. After induction (37 °C), activity of the P_{AS2} promoter goes down while the other three promoters (P_{AS1}, P_{AS3} and P_{AS4}) become active. As shown in Scarlato et al. (1991), at 37 °C, P_{AS1} shows maximal level of activity compared to P_{AS3} and is on within <10 min of induction. The amount of transcripts generated from P_{AS3} is very low and have been reported to be hardly detectable. The P_{AS4} promoter shows same level of activity as P_{AS1} but produces antisense RNA. Although activity of P_{AS4} promoter and its product, the antisense-RNA, is known, the target of the antisense RNA is not known till date. In passing it is important to mention that multi-promoter activity in the operon of TCS as observed in *B. pertussis*, has also been observed in other human pathogens (Chauhan and Tyagi, 2008; Donà et al., 2008).

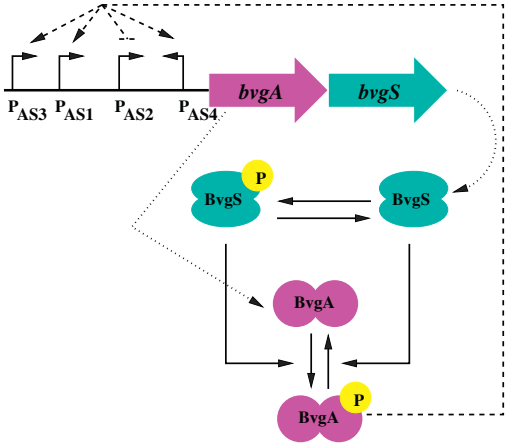

Fig. 1. Schematic presentation of *bvg* locus and signal transduction in BvgAS two component system. The dashed line presents the feedback by phosphorylated dimer of BvgA on its own operon. The dotted line is for the production of dimers of BvgS and BvgA. For simplicity the mRNAs are not shown in the diagram.

To study functioning of the *bvg* locus we consider only the activity of two the promoters P_{AS1} and P_{AS2} in our model, as reasonable amount of experimental data is available in the literature for these two promoters (Scarlato et al., 1991). Both these promoters are typical example of tandem promoter, containing a conserved region of ≈10 base pairs between upstream of P_{AS1} and transcriptional start site (TSP) of P_{AS2}. In the model, we designate the constitutive form of P_{AS2} under non-inducing condition as P_{AS2c}. Once induced, TF interacts with both P_{AS1} and P_{AS2} and makes them active

$$P_{AS2c} + A_{2P} \underset{k_{u2}}{\overset{k_{b2}}{\rightleftharpoons}} P_{AS2a}, \tag{1}$$

$$P_{AS1i} + A_{2P} \underset{k_{u1}}{\overset{k_{b1}}{\rightleftharpoons}} P_{AS1a}. \tag{2}$$

In the above equations P_{AS2a} is the active form of P_{AS2}; and P_{AS1i} and P_{AS1a} are inactive and active form of P_{AS1} promoter, respectively. Due to the presence of conserved region of ≈10 base pairs, RNA polymerase (RNAP) for P_{AS1} traversing through downstream of P_{AS1} now interferes with the binding of TF (and RNAP for P_{AS2}) to upstream of P_{AS2} causing transcriptional interference (see Fig. 2) (Buetti-Dinh et al., 2009; Shearwin et al., 2005). During this process P_{AS2} and P_{AS1} act as sensitive and aggressive promoter, respectively. Although a detailed kinetic mechanism of transcriptional interference has been proposed and verified experimentally (Buetti-Dinh et al., 2009), we use the following notion to keep the model simple

$$P_{AS2a} \underset{k_{a2}}{\overset{k_{i2}}{\rightleftharpoons}} P_{AS2i}, \tag{3}$$

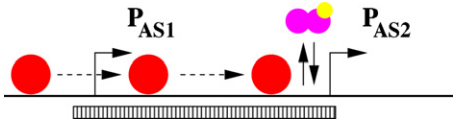

Fig. 2. Schematic presentation of transcriptional interference between P_{AS1} and P_{AS2}. RNA polymerase (red blob) starts its journey from upstream of P_{AS1} promoter executing initiation, elongation and termination (follow the dotted arrowhead). During termination it interferes with the TF (A_{2P}, magenta dimers with yellow blob on top), causing transcriptional interference and downregulation of P_{AS2} promoter activity. The vertical solid arrowheads presents binding/unbinding process between TF and P_{AS2} promoter site. The filled bar at the bottom is for overlapping ≈10 base pair region between upstream of P_{AS1} and TSP of P_{AS2}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)