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Point-cycle bistability and stochasticity in a regulatory circuit for *Bacillus subtilis* competence

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ABSTRACT

Bacillus subtilis is a very well-studied organism in biology. Recent results show that an evolutionary plausible alternative competence regulation circuit for this bacterium, despite presenting equivalent functionality, exhibits physiologically important differences. Thus, it is not *a priori* clear why Nature only selects a specific gene regulation circuit other than a plethora of equivalent others. Here, we use simulations to study this question further. Based on the wild-type *Bacillus subtilis* circuit, we add a positive autoregulation feedback loop to the intermediate gene *comS*. We use bifurcation theory to study the dynamical features of the hypothetical gene circuit versus the feedback strength of the added loop, and we rely on stochastic simulations to perform *in silico* experiments. We discover the existence of a bistable system: a stable limit cycle and a stable fixed point separated by an unstable limit cycle with a varying height of underlying stochastic potential. This structure is absent from the wild type. The coexistence of the unstable limit cycle with stochastic noise endows the circuit with an ability to confine, prevent or switch between its two stable attractors.

1. Introduction

Bacteria may exhibit phenotypic heterogeneity not only based on varying and adaptive genetic contents, but rather, on epigenetic multi-stability – typically bistability – derived from specific feedback processes that underlie gene expression networks [1].

It has been shown mathematically that positive nonlinear feedback regulation is a requirement for the experimental observation of bistability and for excitability [2]. These behaviors are well understood in terms of the underlying nonlinear dynamics of gene regulation [3].

Two famous examples of epigenetic phenotypic variation are the lactose utilization [4–6] and lysis/lysogeny switches [7,8] in *Escherichia coli*. These examples highlight the level of understanding, provided by theoretical modeling and simulations, on the question of the possible contribution of biochemical noise. In the lactose utilization switch, a dynamical phase plane analysis [3,9] delineates the realm of bistability and graded response [10,11]. The stochastic aspects in experiments have been studied by Elowitz et al. [8] and Cai et al. [7]. Evolutionary implications have been theoretically studied by van Hoek and Hogeweg [12] and Santillan [13]. In the lysis/lysogeny switch [14–16], mathematical continuation analysis [9] helped understand regulation [14] and stability [17]. The role of noise has been investigated by Arkin et al. [18] and others [19,20]. A first-principle chemical master equation approach [21,22] is impossible but its numerical solution using the Gillespie algorithm and its descendants [23,24] was used by Arkin et al. [18] to demonstrate the split of one population into two subpopulations. Thus, mathematical analysis is essential to understand the intricate interplay of regulation and biochemical noise in establishing phenotype.

Here, we are concerned with the competence phenotype in the bacterium *Bacillus subtilis*. Competence for transformation is a transient natural ability identified in several species of bacteria, to perform horizontal gene transfer. It is a regulated cellular state induced by environmental conditions whereby bacteria actively accept exogenous free DNA from the environment, and heritably incorporate it into the bacterial genome [25]. Bacterial cells therefore respond to external stimuli to switch on an array of regulatory genes and their associated molecular machinery to accept the DNA. Not all cells in a bacterial population become competent however; the fraction varies with species. *Bacillus subtilis* has one of the highest transformation frequencies in prokaryotic species. Isogenic populations of *Bacillus subtilis* exhibit two states and are therefore bi-stable (in the experimental sense). Between 10% and 25% of a wild-type *B. subtilis* culture exhibits competence [25], but this depends on conditions.

In *Bacillus subtilis*, the master regulator of the competence machinery is the transcription factor ComK [26] that activates well

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over 100 genes [27]. ComK is activated via quorum sensing and it is multiply regulated at the transcriptional level. ComK binds DNA as a tetramer [28] to act as a transcription factor for its own gene. This defines an auto-positive feedback loop at the core of the competence network. As ComK accumulates over competence threshold, it is also being degraded proteolytically. The constitutively abundant protein MecA binds free ComK. The complex is then processed by ClpP–ClpC proteases that degrade the bound ComK, and return the MecA to the system. Degradation of ComK takes the system out of competence. However, the small protein ComS, also induced by the quorum sensing machinery, competes with ComK for binding to MecA. This results in ComK being available again to positively regulate its own transcription. Thus, a buildup to competence can occur again [29–32].

Transcriptional, rather than translational noise, is the main source of noise in gene regulatory systems [8]. Intrinsic stochastic fluctuations (noise) in transcription of the *comK* gene consistent with observed low mRNA transcript numbers induce randomness in the timing of competence excursions [33]. In [34,35], Süel et al. showed how entry into competence is a stochastic event that depends on intrinsic noise and that competence excursions (entry followed later in time by concomitant exit) are a consequence of excitability, a mathematical characteristic of the nonlinear dynamical system defined by the underlying biochemical circuit. They abstracted complex regulatory dynamics to a core of necessary and sufficient processes: positive auto-regulatory feedback of ComK onto its own gene, and indirect negative feedback by ComS on the degradation of the master regulator ComK (see Fig. 1A).

In the Süel et al. model, *Bacillus subtilis* resides mostly in the vegetative state at high ComS and low ComK, randomly fluctuating about in the basin of attraction of an upper stable fixed point of the two-dimensional ComK–ComS regulatory phase plane. Occasionally, random biochemical fluctuations nudge the system to cross the separatrix causing it to undergo a then committed excursion past the middle saddle point and around a lower ComS, higher ComK, unstable spiral fixed point, back into vegetative state, and poised to repeat the process. The Süel et al. model was validated in several ways, and it provides a consistent prediction of the probability of competence initiation, duration and variation thereabout [34,35].

Most recently [36], Cagatay et al. showed experimentally and theoretically, that a subtly different competence circuit topology results in biologically important different noise characteristics. Specifically, model predictions for a mutant *B. subtilis* for which only the order of the composite negative regulation loop through the intermediary *comS* gene was inverted, were confirmed experimentally. The mutant shows a decreased level of noise and behaves more like a regular clock, than an excitable system. It is less efficient at sampling a randomly changing environment making it less desirable than WT, from an evolutionary standpoint.

In the work presented below, we begin reporting on our ongoing survey of the dynamical and stochastic features of evolutionarily plausible alternative core regulation circuits for the phenotype of competence in *Bacillus subtilis*. Herein, we added autoregulation to ComS in the WT regulation scheme, and we named the hypothetical gene and its protein, ComS*. We call this circuit CircuitOne (see Fig. 1B). The dynamic behaviors found in CircuitOne belong to well-known bifurcations types [9]. The stochasticity is due to the randomness of biochemical processes. This noise is purely intrinsic, and it arises only from the fluctuations due to the small numbers of some (or all) chemical reactants in the system [23,37–39]. We hope that our studies will help shed light on how the interplay of dynamics and stochastics impacts evolutionary choices.

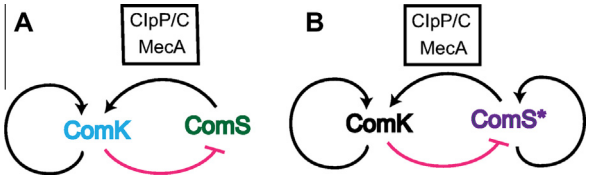

Fig. 1. WT and hypothetical competence diagrams in Bacillus subtilis. (A) Diagram of the WT circuit of competence in Bacillus subtilis. (B) Diagram of CircuitOne. This hypothetically and evolutionarily plausible circuit is similar to WT but has, in addition, positive autoregulation of the intermediate gene (*comS**).

The paper is organized as follows: In Section 2 we present our mathematical methods. In 2.1, we describe the models. In Sections 2.2, 2.3 and 2.4, we explain the deterministic and stochastic viewpoints and their correspondence, respectively. In Section 3, we present our results. In Section 3.1 we study the effect of the coupling strength, and in Section 3.2 we study the effect of different molecular numbers. In Section 4, we discuss our results. In 4.1 we discuss the importance of added feedback, in 4.2 we discuss limit cycles and molecular numbers, and in summary, we discuss our results in light of our motivations for this study.

2. Methods

2.1. Descriptions of the model

2.1.1. Biochemical model

Below, we list all the biochemical reactions of the discrete event model. State variables in the model describe the number of reactant molecules. The first group of reactions is:

$$P^{const}_{comK} \xrightarrow{k_1} P^{const}_{comK} + mRNA_{comK},$$
$$P_{comK} \xrightarrow{f(\cdot)} P_{comK} + mRNA_{comK},$$
$$mRNA_{comK} \xrightarrow{k_3} mRNA_{comK} + ComK.$$

These three reactions describe constitutive and regulated expressions of the *comK* gene. P^{const}_{comK} is the number of constitutive promoters in the system, and P_{comK} is the number of regulated promoters in the system. The number of messenger RNA molecules in the system is denoted by $mRNA_{comK}$. Biochemical events occur at fixed or regulated rates. In the first reaction, k_1 is therefore the unregulated basal transcription rate of the *comK* gene. In the second reaction, the promoter regulation function of the *comK* gene is

$$f([ComK], k_2, k_k, n) = \frac{k_2[ComK]^n}{k_k^n + [ComK]^n}$$

where $[ComK]$ is ComK concentration, and k_k is the ComK protein level when half *comK* genes are activated; k_2 is the coupling strength of *ComK* to its own gene. This reaction describes regulated transcription of the *comK* gene. In the third reaction, k_3 is the rate of translation of messenger RNA into ComK protein.

$$P^{const}_{comS^*} \xrightarrow{k_4} P^{const}_{comS^*} + mRNA_{comS^*},$$
$$P_{comS^*} \xrightarrow{g(\cdot)} P_{comS^*} + mRNA_{comS^*},$$
$$mRNA_{comS^*} \xrightarrow{k_6} mRNA_{comS^*} + ComS^*.$$

The above three reactions are the counterpart triplet of reactions for the *comS** gene. The *comS** promoter regulation function is

$$g([ComK], k_5, k_s, p, [ComS^*], b_{ss}, s_s, n^*) = \frac{k_5 k_s^p}{k_s^p + [ComK]^p} \cdot \frac{b_{ss}[ComS^*]^{n^*}}{s_s^{n^*} + [ComS^*]^{n^*}},$$

where $[ComS^*]$ is *ComS** concentration, k_s is the ComK protein level when half *comS** genes are activated, and s_s is the ComS protein level when half *comS** genes are activated; k_5 and b_{ss} are coupling