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Subtle interplay of stochasticity and deterministic dynamics pervades an evolutionary plausible genetic circuit for *Bacillus subtilis* competence

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ABSTRACT

Here we study the interplay of stochastic and deterministic dynamics in an evolutionary plausible candidate core genetic circuit for *Bacillus subtilis* competence. We find that high noise would not necessarily be detrimental to the circuit's ability to deliver the phenotype, due to an unexpected built-in robustness that we further investigate. Also, we find that seemingly subtle deterministic dynamical features of the regulation, unstable and stable limit cycles, while in the presence of biochemical noise, would result in a distinctive new observable in the phenotype. We conduct mathematical analyses of the system's stability at the fixed points and derive some general model-independent consequences. We also show how imperfect time-scale separation in the system would result in observables detrimental to the phenotype, that nature could have harnessed for selection.

1. Introduction

Bacillus subtilis is a common bacterium found in the ground. Evolution has endowed this bacterium with the ability to evolve using an epigenetic adaptation mechanism called competence. Competence is the process by which individual cells of a bacteria population under stress may accept exogenous DNA fragments randomly present in that environment, and incorporate the new genetic material into their own genome, attempting to adapt to randomly changing environmental conditions [1–3]. Competence has been studied both experimentally and theoretically very extensively [4,5]. Here we are concerned with why evolution selects some gene regulatory topologies over other functionally equivalent ones. Specifically, we are interested in determining the impact on such choices by the subtle interplay of stochasticity and deterministic dynamics exhibited by candidate alternative gene regulatory topologies. Recently, much light has been shed on this issue by Süel et al. in [6] where it was predicted theoretically and confirmed experimentally, that functionally equivalent gene regulatory topologies actually differ in their stochastic behavior substantially such that it is plausible to suggest that the main reason why evolutionary selection of one regulation topology over another is actually the subtle difference in stochastic behavior.

Core gene regulation of wild-type competence involves the interplay of only two genes, *ComK* and *ComS*. *ComK* is the master gene regulator. When it is up-regulated, competence is enabled. *ComK* positively directly auto-regulates itself. But it also indirectly negatively regulates itself. It does the latter via an intermediary gene called *ComS*. In nature, *ComK* negatively regulates *ComS* first and, indirectly, *ComS* then positively regulates *ComK*. The net effect is negative indirect regulation. The authors of [6] discovered that if the order of the negative regulation is reversed such that positive regulation of the intermediary gene occurs first, and negative regulation of *ComK* follows next, thus implementing an equivalent effective negative indirect regulation of *ComK*, the resulting circuit is still functional, but the noise characteristics are no longer ideal to effectively support the competence phenotype. The authors of [6] found that, compared to wild-type, the resulting circuit's fractional variation of time durations of its competence events (σ/μ) is too small. In short, the circuit is too precise. It behaves much more like a regular biological clock with which it shares core regulatory topology. Hence, the shapes of the distributions of time spent in the competent state differ: the wild-type time in competence distribution presents a long extended high tail, the mutant circuit does not. The reverse-order regulation mutant in fact fails to implement an ideal competence phenotype; in summary this mutant circuit breaks the phenotype. The authors of [6] show that regularly (i.e., with nearly unique frequency) sampling an otherwise randomly changing environment results in inefficient competence. Of course, the alternative gene circuit studied by the authors of [6] is only one of a plethora of others evolution may have considered. We may then ask: what does the subtle interplay of stochasticity and deterministic nonlinear dynamics of gene regulation have to say about evolutionary choices?

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Here, we report the results of our study of this question in the context of the core gene regulatory circuit of *B. subtilis* competence described by Fig. 1. The circuit studied here arises naturally from a plausible evolutionary hypothetical modification of a circuit already studied extensively both theoretically and experimentally in [6]. The core regulatory elements are the same. In addition however, our circuit, which we call CircuitTwo, includes an auto-regulation loop on the intermediate gene we called *MecA** to differentiate it from the *MecA* of [6]. This loop is absent in the SynEx mutant of [6]. We found CircuitTwo to present a number of subtle nonlinear dynamical features and we found the phenotype to be profoundly influenced by the interplay with intrinsic biochemical noise. CircuitTwo is so-named because it is the second circuit we studied in a series of evolutionary plausible variants of wild-type core genetic regulation of *B. subtilis*.

In this work, we build on modeling and analyses developed in [5]. Briefly, there, the question investigated was whether and how inverting the order of the overall negative compound regulation of *ComK* onto itself via the intermediate gene *ComS* would matter to phenotype. Un-intuitively, it was found that it is the noise characteristic of the circuit that was most profoundly impacted. Thus, a seemingly innocuous dynamical change — order inversion — that could be argued to deliver no net functional change, instead was found both theoretically and confirmed experimentally, to lead to spoiling the competence phenotype by reducing the variability of competence excursion lengths. Stated differently, the induced effect was to change the shape of the probability density function of competence excursion durations. Specifically the effect is to remove the high tail of the distribution; to tighten it to be more symmetric about the mean. This supports the notion that impact from seemingly trivial changes in the topology of the regulation network may have subtle yet profound biological consequences. Here we have set out to study the consequences of adding positive auto-regulation onto the intermediate gene in the negative regulation loop of *ComK*. While this modification may superficially appear minimal, in fact, we find the resulting impact on the circuit's behavior subtle yet profound, and most importantly, biologically meaningful. In a previous analysis, we studied the consequences of adding positive auto-regulation onto the intermediate gene of the wild-type competence circuit [7]. Our results here complement this study.

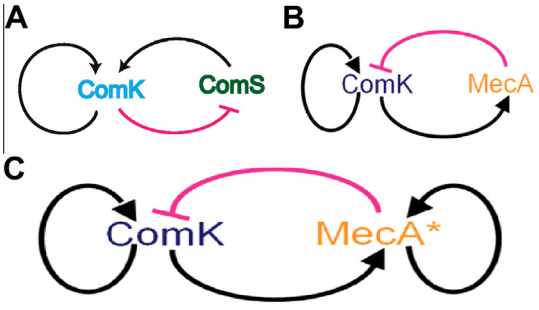

Fig. 1. Core genetic regulation competence circuits for *Bacillus subtilis*. (A) Wild-type (WT) competence regulation. ComK positively auto-regulates itself, but also indirectly affects down-regulation on itself through the intermediate gene *ComS*. Down-regulation is followed by up-regulation. Implied is the ClpP/C protease degradation process which consists of competitive binding by free *MecA* to free *ComK* or to free *ComS* and proteolytic degradation of the bound protein. (B) SynEx mutant regulation of competence. *ComK* positively auto-regulates itself. Contrary to WT however, the order of the net down-regulation of *ComK* through an intermediate gene, here *MecA*, is opposite wild-type. Thus, up-regulation is followed by down-regulation. Through the ClpP/C protease degradation process, *MecA* effectively down-regulates *ComK*. (C) CircuitTwo regulation of competence. Similar to WT and SynEx regulations, *ComK* positively auto-regulates itself. Similar to SynEx, *ComK* effectively down-regulates itself through an intermediate gene, *MecA**. Up-regulation is followed by down-regulation. As in SynEx, through the ClpP/C protease degradation process, *MecA** effectively down-regulates *ComK*. However, different from SynEx, *MecA** is endowed with positive auto-regulation.

2. Results

2.1. CircuitTwo from the stochastic and deterministic viewpoints.

CircuitTwo was built using SynEx [6] as a template. The biochemical event representation is given in Methods A. At the most basic level suitable for stochastic simulation using the Gillespie algorithm [8,9], the model includes dynamical variables for the two proteins in the system *ComK* and *MecA**, parameters for the basal and regulated promoters of *ComK*, $P_{constComK}$ and P_{ComK} respectively, and of *MecA**, $P_{constMecA^*}$ and P_{MecA^*}. The model also includes dynamical variables for RNAs of *ComK* and *MecA**, $mRNA_{ComK}$ and $mRNA_{MecA^*}$. In the stochastic description, the dynamical variables represent copy numbers of molecules. All parameters of the full stochastic model are listed in Table 1.

From the biochemical event level description, we derived the deterministic ordinary differential equation based model described in Methods B. Since the number of promoters in the system is constant, there are only four dynamical variables: *ComK*, *MecA**, $mRNA_{ComK}$ and $mRNA_{MecA^*}$. We call this continuous model the 4-ODE model. Because the dynamics of this model presents a slow and fast manifold, we have derived a reduced version of the model in which the dynamics of the messenger RNAs is assumed to be constant. This is shown in Methods C. We call this model the 2-ODE model and the procedure to obtain it an adiabatic approximation, also known as QSSA [10,11]. Details are in Appendix A. In the deterministic descriptions (2-ODE and 4-ODE) all dynamical variables are in units of concentration (nM). The parameters of the 4-ODE model are the same as the stochastic model (Table 1). Note: we divide by the volume of the cell (Ω) to obtain concentrations. The parameters of the 2-ODE model are listed in Table 2.

In the limit of infinite number of molecules, known as the *thermodynamic limit*, the average behavior of the biochemical discrete event level model is mathematically expected to coincide with the predictions of the 4-ODE deterministic model [12]. However, only in the limit of infinite time-scale separation between the slow and fast manifolds, may we expect the 2-ODE model to be in exact agreement with the 4-ODE model.

2.2. A workable competence regime in the presence of only one stable fixed point with no local rotation

Our initial investigation of CircuitTwo is based on the parameters of [6] and estimated parameters for added positive auto-feedback loop onto *MecA**. Because strengthened *MecA** induces more suppression of *ComK* than in SynEx, we adjusted the parameters of the coupling at *MecA** such that the resulting phase portrait is similar to the SynEx mutant of [6]. As discussed in Methods C, the coupling at the *MecA** is controlled by the following regulation function:

$$g^*(\cdot) = k_4 \cdot \frac{\beta_1[K]^p + \beta_2[M]^{n^*} + \beta_3[K]^p[M]^{n^*}}{k_m^{p\cdot n^*} + \beta_1[K]^p + \beta_2[M]^{n^*} + \beta_3[K]^p[M]^{n^*}}, \quad (1)$$

where $[K]$ ($[M]$) represents the concentration of *ComK* (*MecA**), and other parameters are listed in Table 1.

We were particularly interested in the behavior of the model as a function of the parameter k_m because it causes the *MecA** nullcline to shift horizontally on the *ComK*-*MecA** phase plane. This behavior is similar to the effect caused by changing the Michaelis constant in classical Michaelis–Menten enzyme kinetics, which would shift the enzymatic reaction rate curve horizontally by