Transcriptional regulation on the expression of the master regulator, Spo0A

1 Introduction

Under starvation conditions, the soil bacterium *Bacillus subtilis* initiates entry into either a unicellular (sporulation) or a multicellular (biofilm) differentiation pathway for survival. These differentiation programs require the activation of a master regulatory transcription factor Spo0A. The expression of Spo0A is known to be controlled by a transcriptional feedback loop. The *spo0A* gene is transcribed from two distinct promoters, Pv and Ps (Fig. 1A). Pv is recognized by σ^A-RNA polymerase and is considered to be active during vegetative growth, while Ps is controlled by σ^H-RNA polymerase and is induced during the sporulation process[1, 2, 3]. During the transition from the vegetative growth to the sporulation process, the Pv promoter is repressed and the Ps promoter is activated; this process is known as a promoter switching [3].

The direct binding of Spo0A∼P to its own promoter is considered to be the main reason of the promoter switching process[4, 3]. There are four different binding sites of on the *spo0A* promote region, labeled as boxes 0A1 - 0A4 (Fig. 1A). When nutrients are sufficient for vegetative growth, the upstream Pv promoter is transcribed by σ^A-RNAP and the downstream σ^H-RNAP dependent Ps promoter is repressed by relatively low levels of Spo0A∼P bound to the 0A2 box [5, 6, 2, 3](Fig. 1B). During starvation, relatively high levels of Spo0A∼P can bind to the 0A3 box, antagonize the Spo0A∼P bound to the 0A2 box and induce the transcription from the Ps promoter[3, 6](Fig. 1B). Under such conditions, the upstream Pv promoter is repressed by Spo0A∼P bound to the 0A1 box [3]. Notably, though the Ps promoter is recognized by σ^H-RNA polymerase, the rising of σ^H level is considered to be not the main reason of the activation of Ps [4].

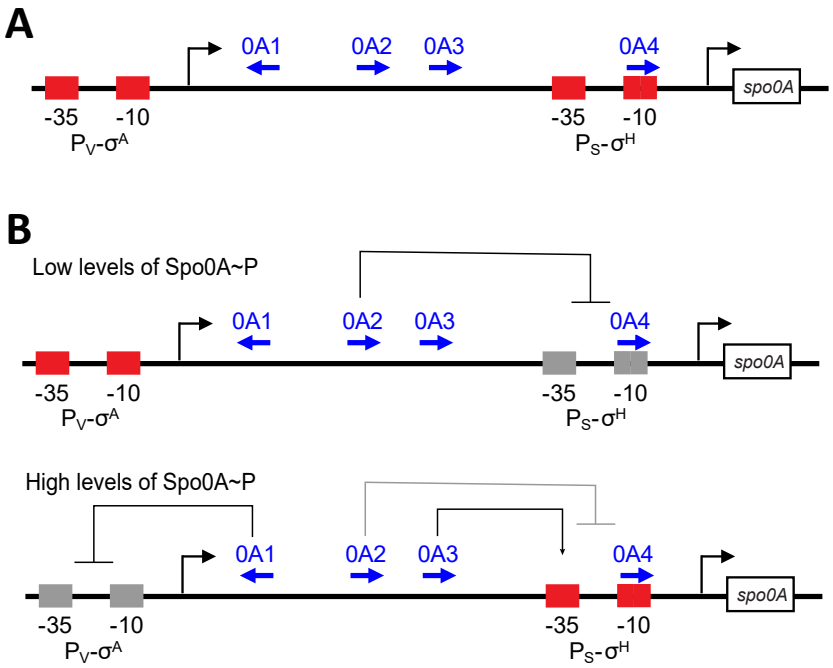

Figure 1: The model of the transcriptional regulation of *spo0A* promoter. (A) The structure of the promoter of *spo0A*. The blue arrows show the different binding boxes. (B) The traditional model of the transcriptional regulation of *spo0A* promoter.

2 Preliminary results that question the traditional model

2.1 The activation of Ps does not require the binding of Spo0A∼P on the 0A3 box

To explore the role of different binding boxes in the regulation of the *spo0A* promoter activity, we constructed pure Ps and Pv promoters by removing the core region of Pv and Ps, respectively. The combinations of the mutations on 0A1 - 0A3 boxes were then introduced to the isolated Ps and Pv promoters. These combinations of mutations are denoted as 1*, 2*, 3*, 1*2*, 1*3*, 2*3*, and 1*2*3* (you can check Masaya's slides for the promoter constructions). Here 1* means that the 0A1 box is mutated; 1*2* means that 0A1 and 0A2 boxes are mutated; etc. The mutated binding boxes can no longer be bound by Spo0A∼P.

Since 0A4 is overlapping with the -10 region of the Ps promoter, we did not test mutations on the 0A4 box. A *lacZ* reporter was used to measure the activities of these promoter constructs.

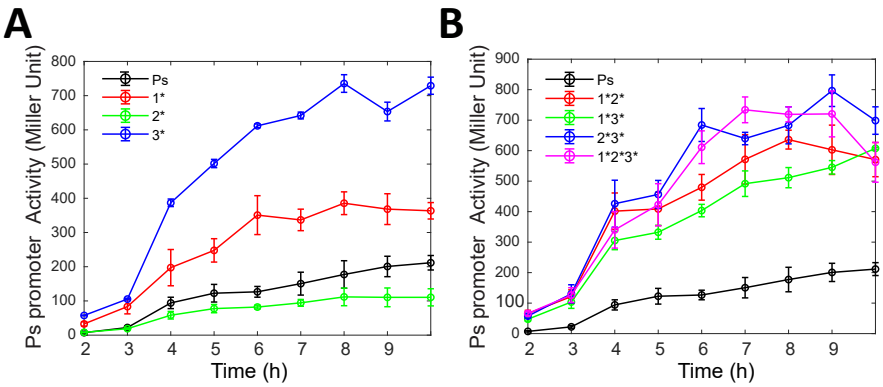

Figure 2: The dynamics of Ps activity with different combinations of mutations on the Spo0A∼P binding boxes. The black lines correspond to the Ps promoter that contains no mutation on the Spo0A∼ binding boxes. (A) The dynamics of the original Ps promoter and the promoters with the mutations 1*, 2*, and 3*. (B) The dynamics of the original Ps promoter and the promoters with the mutations 1*2*, 2*3*, 1*3*, and 1*2*3*.

As Fig. 2A shows, the mutations on 0A3 and 0A1 boxes would increase the activity of the Ps promoter. In contrast, the mutation on 0A2 resulted in lower Ps activity. These results suggest that the 0A2 binding box has a positive effect on the activity of the Ps promoter, while the 0A1 and 0A3 boxes have negative effects. The results conflict with the traditional model (Fig. 1B), in which the 0A2 binding box is hypothesized to be repressing Ps under low levels of Spo0A∼P [3]. Moreover, as Fig. 2B shows, the Ps promoter activity was increased when two or more binding boxes were mutated. These results show that the activation of the Ps promoter at late times does not require the binding of Spo0A∼P on its own promoter, which is also in conflict with the traditional model[4, 3]. Moreover, the dynamics of Ps activity with 1*2*3*, 1*2*, 1*3*, and 2*3* mutations were relatively similar. These results indicate that every binding box cannot individually repress Ps, which suggests some complex mechanisms that control the Ps promoter activity.

2.2 Pv is mainly repressed by the binding of Spo0A∼P on the 0A3 box

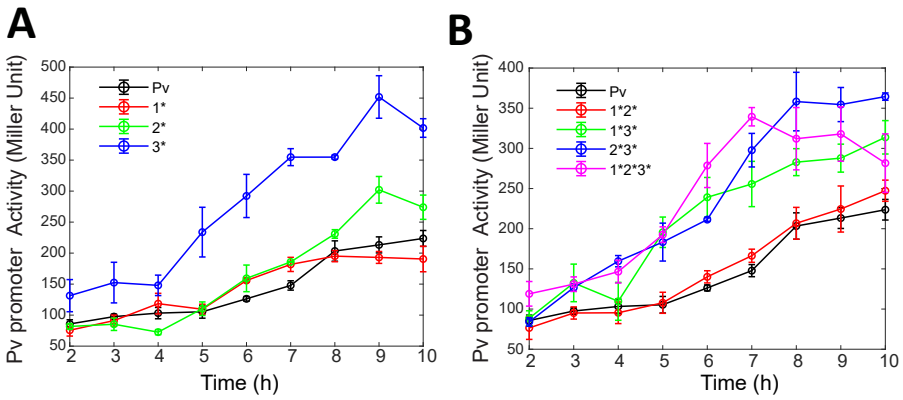

Figure 3: The dynamics of Pv activity with different combinations of mutations on the Spo0A∼P binding boxes. The black lines correspond to the Pv promoter that contains no mutation on the Spo0A∼ binding boxes. (A) The dynamics of the original Pv promoter and the promoters with the mutations 1*, 2*, and 3*. (B) The dynamics of the original Pv promoter and the promoters with the mutations 1*2*, 2*3*, 1*3*, and 1*2*3*.

As to the Pv promoter, our results show that the mutation on the 0A3 box has a positive effect on the activity of Pv, while mutations on the 0A1 and 0A2 boxes have relatively minor effects (Fig. 3A). As Fig. 3B shows, the 1*2* mutation did not change the dynamics of Pv activity significantly, while all other mutations could increase the activity of Pv. These results suggest that the Pv promoter is mainly repressed by the 0A3 box, which is also in conflict with the traditional model in which Pv is repressed by the 0A1 box [3] (Fig. 1B). Moreover, we did not observe a decrease in the Pv activity at late times. Thus, we need a new model to explain these experimental observations.

2.3 Thermodynamic modeling of the *spo0A* promoters

To illustrate the feedback regulation of *spo0A* transcription, we constructed a thermodynamic model of the *spo0A* promoters. Here we assume that each box can be only bound by one Spo0A∼P dimer($(\text{Spo0A}\sim\text{P})_2$), so the binding of Spo0A∼P on the binding boxes 0A1, 0A2, and 0A3 has 8 configurations, labeled as $\{0, 1, 2, 3, 12, 13, 23, 123\}$. Here 0 means no binding box is occupied by Spo0A∼P, 1 means the 0A1 box is occupied by Spo0A∼P, etc. Consider the binding of RNA polymerase (RNAP) on the promoter, the promoter Pv or Ps has 16 states, and the probability of each state is given by:

$$P_{i,r} = \frac{exp(-\frac{\varepsilon_{i,r}}{kT})[(Spo0A \sim P)_2]^{n_i}[RNAP]^{n_r}}{\sum_i \sum_r exp(-\frac{\varepsilon_{i,r}}{kT})[(Spo0A \sim P)_2]^{n_i}[RNAP]^{n_r}} \quad (1)$$

Here i is in $\{0, 1, 2, 3, 12, 13, 23, 123\}$, representing the configuration of the binding of Spo0A∼P. r is in $\{0, 1\}$, representing the configuration of the binding of RNAP. n_i and n_r represent the number of the molecules of $(\text{Spo0A}\sim\text{P})_2$ and RNAP bound to the promoter, respectively. $\varepsilon_{i,r}$ represents the energy of the state {i,r}. Following [7], we assumed that the maximum initiation rate of the transcription is not changed with the binding of Spo0A∼P. On the other hand, the binding of Spo0A∼P regulates the transcription rate mainly by changing the binding affinity of RNAP, i.e., the energy $\varepsilon_{i,r}$. So the total transcription rate is given by:

$$v_{tot} = \sum_i v_{max} \cdot P_{i,1} \quad (2)$$

$$(3)$$

Here v_{max} is the maximum transcription rate. Note that the transcription only starts when RNAP is bound to the promoter, so only the states with $r = 0$ were counted. Following [4], we assumed that the concentrations of the sigma factors are constant, so the [RNAP] is constant.

3 Future directions

We got some new data from Dr. Masaya Fujita. The data contains the measurements of the binding affinity on different Spo0A∼P binding boxes, and the (updated) dynamics of the promoters carrying different mutations.

(1) Add new restrictions to the model based on the binding affinity of each binding box.

(2) Using this model to can calculate the activity of *spo0A* promoters as functions of time. Basically, we can use a Spo0A∼P dynamics(a function describing how [Spo0A∼P] increases with time) predicted by the model of the phosphorelay network as the input, and calculate the promoter activity as a function of time. Then we can fit the unknown parameters(e.g. the energy of each state) to the experimental data(perhaps with a particle swarm algorithm). The preliminary result(Fig. 2,3) might be incorrect; please use the new data.

(3) Check the cross-talk between Pv and Ps. The simplest hypothesis is that the transcription from Pv and Ps are independent from each other; if this is not correct, we need to consider to change the model to explain the interaction between Pv and Ps.

(4) Alternative assumptions. For example, we assumed that the [RNAP] is constant. However, the concentration of σ^H and σ^A may also change with time so the [RNAP] could also be a function of time. We may also need to consider the binding of [Spo0A∼P] on 0A4 box.

(5) Experimental directions: To understand how the dynamics of cellular Spo0A∼P level affects the expression of *spo0A* gene, we may want to measure the activity of *spo0A* promoters in $\Delta kinA$ and Δsda(in which the [Spo0A∼P] dynamics is perturbed) backgrounds. Moreover, to understand the role of the feedback regulation of the transcription of *spo0A*, we may want to measure the expression of biofilm and sporulation genes in the strains harboring different mutations on the *spo0A* promoter. We expect to get more experimental data to further calibrate the model.

References

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