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0.1 Description of regulatory network

In figure 1, we present a simplified gene regulatory network that describes the interactions between the master regulator Spo0A, and the biofilm regulatory proteins SinI, SinR, and SlrR.

Surfactin is known to indirectly activate the expression of genes that produce matrix, although the precise molecular process is not fully understood. It has been suggested that surfactin creates pores in the membrane, leading to the leakage of potassium ions from the cytoplasm to the outside of the cell. This causes the activation of the membrane sensor kinase KinC when potassium levels are low, which then phosphorylates the master gene regulator Spo0A[20]. However, some studies contradict this exact mechanism.[21] Another mechanism responsible for indirectly activating the matrix-producing genes is nutrient depletion, by which kinases (such as KinA) phosphorylate Spo0A, which can then activate the transcription of SinI.

When Spo0A is activated (phosphorylated), it triggers the transcription of the SinI protein. The SinI protein binds to the SinR protein to form a SinI-SinR complex, which effectively titrates SinR, reducing the number of freely available SinR proteins. SinR is a protein that suppresses the transcription of genes related to matrix production. By disabling SinR through the formation of the SinI-SinR complex, the cell can activate the genes responsible for matrix production.[18]

Furthermore, SinR also inhibits the expression of another protein known as SlrR. If SlrR is expressed, it can also suppress SinR by interacting with it to form a SlrR-SinR complex.[18]

Spo0A has a self-regulatory mechanism that allows it to activate or repress its own transcription depending on the concentration of its phosphorylated form. When Spo0A~P is absent, the gene *spo0A* is transcribed at a basal low-rate during vegetative growth; this transcription is mediated by the housekeeping sigma factor σ^A and the promoter P_V . At non-zero low concentrations of Spo0A~P, the transcription of *spo0A* is further activated with the help of the P_S promoter and the σ^H factor. At these low concentrations, Spo0A~P activates biofilm formation and the expression of toxins involved in cannibalism. If the concentration of Spo0A~P increases further, it will reach high levels, which will lead to the repression of the *spo0A* gene and *sinI* gene transcription, while activating the transcription of genes responsible for sporulation.

$$f(A) = \left(\frac{(A/K_A)^{n_A}}{1 + (A/K_A)^{n_A} + \frac{A^2}{K_A K_S}} \right)$$

$$g(R) = \left(\frac{1}{1 + (R/K_R)^{n_R}} \right)$$

where A represents the concentration of Spo0A~P in the cytoplasm. K_A and K_R represent the binding affinities of Spo0A and SinR, respectively, while n_A and n_R represent the cooperativities of the binding. K_S is a constant that accounts for the inhibition of Spo0A~P when it reaches high concentrations.

Equation (1) describes the dynamics of the SinR protein concentration in the cell. SinR is produced at a basal rate P_3 , but its concentration is reduced by a combination of degradation and dilution at a rate D_R due to cell growth, or increased by the activation mediated by low levels of Spo0A~P. Additionally, the concentration of SinR is affected by its interaction with SinI and SlrR, which form complexes with SinR at rates $K_{on(RI)}$ and $K_{on(RL)}$, respectively, leading to a reduction in free SinR molecules.

Equation (2) represents the changes in the concentration of SinI. SinI is produced according to its own maximum production rate P_1 , which is modulated by the function $g(R)$ that models SinR's repression, and the function $f(A)$ which accounts for activation by low levels of Spo0A~P and repression by high levels of Spo0A~P. Just like SinR, the concentration of SinI is decreased both by degradation and dilution at a rate of D_I , and by its binding to SinR, forming the RI complex at a rate of $K_{on(RI)}$.

Equation (3) represents the dynamics of the SlrR protein concentration. The synthesis of SlrR is again dependant on a production rate (P_L), modulated by the function $g(R)$ representing the repressive effect of SinR on SlrR transcription. Similarly, it also considers SlrR's degradation and dilution through the rate D_L and its interaction with SinR, which sequesters SlrR into the RL complex at the rate $K_{on(RL)}$.

Lastly, (4) describes the rate of change of the concentration of Spo0A~P. Spo0A~P is produced at a basal rate P_V and at an activated rate P_S when the concentration of Spo0A~P is low. For simplicity, we assume that the phosphorylation of Spo0A is carried out at a constant rate, which allows us to ignore the concentration of the unphosphorylated form of Spo0A. However, this should be taken into account when considering the extracellular concentration gradients of nutrients and surfactin.

The Hill function and Haldane equation represent the regulatory effects of two proteins, Spo0A and SinR, in a phenomenological manner. The Haldane equation $f(A)$ models the phenomenon in which Spo0A promotes gene transcription when its concentration is low, but inhibits the same transcription when its concentration is high. On the other hand, the repression Hill function $g(R)$ describes how SinR inhibits the expression of genes that produce SlrR and SinI. As the concentration of SinR increases above a threshold (K_R), the level of repression becomes significant. The Hill functions mathematically represent the biological processes of gene activation and repression in a sigmoidal, threshold-dependent manner.

The system of differential equations just presented is largely similar to the one proposed by Dannenberg et al. [22]. The main difference is that, unlike Dannenberg et al., this work will

consider the inhibitory effect of SinR on SinI transcription as well as the inhibitory effect of Spo0A~P at high concentrations.

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