Abstract

Under conditions of nutrient limitation, *Bacillus subtilis* cells terminally differentiate into a dormant spore state. Progression to sporulation is controlled by a genetic circuit structured as a phosphorelay embedded in multiple transcriptional feedback loops, and which is used to activate the master regulator Spo0A by phosphorylation. These transcriptional regulatory interactions are "bandpass"-like, in the sense that activation occurs within a limited band of Spo0A$\sim$P concentrations, and have recently been shown to pulse in a cell-cycle-dependent fashion. Additionally, the core phosphorelay is an architectural variant of the canonical two-component signaling system, which allows signal integration from a larger number of inputs, including two types of phosphatases that act on different protein components. However, the impact of these pulsed bandpass interactions on the circuit dynamics preceding sporulation and the utility of two types of phosphatases remains unclear. In order to address these questions, we measured key features of the bandpass interactions at the single-cell level and analyzed them in the context of a simple mathematical model. The model predicted the emergence of a delayed phase shift between the pulsing activity of the different sporulation genes, as well as the existence of a stable state, with elevated Spo0A activity but no sporulation, embedded within the dynamical structure of the system. To test the model, we used time-lapse fluorescence microscopy to measure dynamics of single cells initiating sporulation. We observed the delayed phase shift emerging during the progression to sporulation, while a re-engineering of the sporulation circuit revealed behavior resembling the predicted additional state. The core phosphorelay model also showed a post-translational bandpass response, and we find that the two types of phosphatases can independently tune the two bandpass thresholds. These results show that periodically-driven bandpass feedback loops can give rise to complex dynamics in the progression towards sporulation, and that similar inputs can tune different response features.