Chapter 4

Conclusion

4.1 Summary of Contributions

Biological processes, spanning scales from individual proteins to entire ecosystems, exhibit significant temporal organization. Widely occurring examples at the scale of individual cells include self-repeating cycles of circadian rhythms, and transitions from one state to another observed during differentiation. Explaining how these cellular behaviors are implemented through dynamic interactions between genes and proteins is a fundamental biological question. Analogous to their roles in the analysis and design of temporal patterns throughout engineering and science, methods of dynamics and control have provided significant insight into this problem. However, two recent developments — large-scale characterization of molecular components, and new methods to visualize these interactions over time — present an opportunity to revisit this fundamental problem and offer more complete solutions. Recent examples of this include a study of transient differentiation, where excitable dynamics in the underlying circuit naturally explain the observed pulse-like phenomena [58], and chemotaxis, where the identification of integral feedback control in the molecular circuit is a key design principle in understanding how robust performance arises from inherently noisy components [67].

In this thesis, we study whether and how temporal ordering of events is implemented in terminal differentiation, using the canonical example of sporulation in *B. subtilis*. Progression to sporulation can occur over multiple cell generations, after which an ordered sequence of events remodels the cell into a dormant spore. The underlying molecular circuit, consisting of a phosphorelay embedded in transcriptional feedback loops, has been extensively characterized, and exhibits several features that are striking from a dynamical systems point

of view, including bandpass interactions coupled together in feedback loops, and modulated periodically in a cell-cycle-dependent fashion. However, how these nonlinear time-varying features combine to generate patterns of gene expression during the sporulation progression is unclear.

Here, we have addressed this issue by combining dynamical models with single-cell time-lapse fluorescent microscopy techniques. We have characterized the bandpass interactions between circuit components. Based on these measurements, we have constructed a simple mathematical model and analyzed it under conditions of periodic input, which mimic cell-cycle modulation effects in real cells. The model predicts the emergence of a delayed phase shift between circuit components, whose existence we verify experimentally. The model also predicts an alternate cellular state that can be accessed using a genetic perturbation, and we have shown that similar behavior can be observed experimentally. Lastly, we have used computational models to explore the significance of the phosphorelay architecture in the context of simpler options available in the superfamily of two-component signaling systems. We have shown how the phosphorelay architecture enables two types of inputs that can independently regulate different aspects of its response, as well as how phosphotransfer building blocks can be used to construct signaling amplifiers.

4.2 Future Work

4.2.1 Systematic perturbation of bandpass responses in sporulation progression

Given that coupled bandpass feedback loops can generate dynamic patterns during sporulation progression, a natural question to ask is how sporulation progression depends on the underlying dynamical patterns and bandpass characteristics. This is important in further understanding how initiation of sporulation depends on the dynamic interactions in the phosphorelay circuit. Addressing this question will require a combined experimental-modeling approach. On the experimental side, a prerequisite is to determine the mechanism of the transcriptional bandpasses. How gene regulation functions are encoded in the DNA sequence of the promoter has been previously investigated in prokaryotic contexts (lac, lambda), and may serve as a useful template for this. Following this, native promoters in the sporulation circuit need to be replaced by promoters with altered bandpass characteris-

tics. The current mathematical model, or more complete versions of it, can serve as a tool to identify which of the bandpass characteristics are most likely to change circuit dynamics and have a significant impact on sporulation progression. A more complete understanding of how sporulation progression depends on the phosphorelay circuit, likely to arise from such an investigation, will be an important step towards understanding similar processes in other bacterial species.

4.2.2 Role of temporally ordered gene expression in formation of the asymmetric septum

In many types of cells, the decision to initiate differentiation is followed by mechanical rearrangements of cellular structures. While dynamic single-cell studies have characterized gene expression during decision-making as well as during mechanical rearrangements, the coordination between these processes is unclear. For example, the first major morphological development after the initiation of sporulation in *B. subtilis* is a switch from medial cell division to the formation of an asymmetric septation. Not only is this switch regulated by the phosphorelay circuit, but it also occurs immediately after initiation. While it has been shown that the phosphorelay circuit can generate temporal order in the activation of target genes, it is not clear if such temporal order is relevant for the formation of the asymmetric septum. Medial cell division involves the formation of a FtsZ ring at the mid-point of the cell. While the transition from medial cell division to asymmetric septation is not completely known, there are several striking features associated with this: One, the medial FtsZ ring translocates to the asymmetric septation at sporulation initiation. Two, this transition can be triggered even in vegetatively growing cells harboring an extra copy of the FtsZ gene by the induction of a FtsZ-associated protein SpoIIE. Three, during sporulation initiation, FtsZ and SpoIIE are upregulated by low and high levels of Spo0A$\sim$P, respectively. We propose to investigate if the phosphorelay circuit encodes the following sequential order of events: First, a translocation of the FtsZ ring away from the medial position. Second, stabilization of the FtsZ ring by SpoIIE at a distal position. To check if this is the case, we will develop a mathematical model of the FtsZ-SpoIIE dynamics and test it with different times of activation of FtsZ relative to SpoIIE. We expect a specific FtsZ-SpoIIE time-lag to be an optimum choice for asymmetric septation, with deviations from this generating altered locations or spurious septation. Furthermore, examination of the underlying mechanisms

may reveal sources of robustness in this process. An alternative possibility is that there is temporal order in this process, but that it is an instance of resource optimization rather than of an operational requirement. Such a study is relevant to other processes where mechanical rearrangements follow initiation, for example in the germinal vesicle breakdown in oocyte maturation [66], or in the formation of whorls during flowering [34]. This study should also shed light on fundamental physical processes of symmetry-breaking, as in which of the two polar positions of the FtsZ ring is eventually chosen, and scaling, as in the positional invariance of the asymmetric septum for different cell sizes that can arise in different environments or in other related species.

4.2.3 Power consumption in two-component signaling architectures

Power consumption is an important constraint in the design of electrical circuits. Typically, performance capabilities of a circuit are limited both by the energy required and the heat generated during operation. Based on operational similarities between 2CS and electrical circuits, power consumption may also be a constraint in the evolution of signaling circuits in cell biology. Phosphorylation-based signaling circuits are ideal for studying this phenomenon, as the identity of the energy source (ATP) is known. Further, the family of two-component signaling circuits presents a diversity of architecture and possibly function that can be used to get a broader perspective on the problem. Therefore, an investigation of how power is consumed across different two-component architectures is likely to yield significant insights into cellular signaling. We propose to approach this problem computationally, using simple models of two-component system architectures to calculate power consumption. Motivated by engineering considerations, we expect power consumption to be directly related to the information transferred, or the bandwidth, of the signaling response. Results from this study are likely to highlight tradeoffs between power consumption and signaling efficacy in cells. Complementarily, how cellular signaling circuits operate under these power constraints may serve as a guiding principle in proposing energy-efficient architectures for use in engineering contexts.