Reverse engineering: the architecture of biological networks

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We adopt a control theory approach to reverse engineer the complexity of a known system—the bacterial heat shock response. Using a computational dynamic model, we explore the organization of the heat shock system and elucidate its various regulation strategies. We show that these strategies are behind much of the complexity of the network. We propose that complexity is a necessary outcome of robustness and performance requirements that are achieved by the heat shock system’s exquisite regulation modules. The techniques we use rely on dynamic computational models and principles from the field of control theory.

Introduction

What lies behind the complexity of biological networks? Can one make sense of the web of interconnections that result from the myriad of molecular interactions within living cells? If one represents the different constituents of a cell (e.g. DNA, RNA, enzymes) as the nodes of a network and then draws a line between any two nodes whose corresponding constituents interact in a significant way, the complexity of the resulting network representation will be daunting. Is this complexity gratuitous— a kind of a historical accident? Are all these connections necessary? And how can one unravel this complexity to understand how these biological networks carry out their function robustly?

In trying to answer these questions, one key observation is that much of the complexity of cellular networks has to do with regulation of cellular function. In fact, regulation is a running theme throughout all of biology. Various strategies for regulation exist, but none is as ubiquitous as feedback. This is not unlike synthetic engineering systems where feedback control strategies can be found universally. Our approach to studying biological complexity is driven by the methods and techniques of control theory: an interdisciplinary branch of engineering and mathematics that deals with the regulation of dynamical systems of any type. Control theoretic approaches have been applied profitably in a wide spectrum of disciplines, including economics, engineering, and the physical sciences. Control theory has had less impact on the biological sciences, although this is beginning to change. In this article, we use a control theory approach to explore the exquisite architecture of a well-known cellular stress system—the bacterial heat shock response. Our goal is to demonstrate the effectiveness of this approach and its potential to generate a deeper understanding of biological complexity.

The heat shock response in bacteria is an important mechanism for combating the stress associated with an increase in temperature in the cellular environment (1). The resulting increased heat causes the unfolding or misfolding of cellular proteins and leads to a state of cellular stress. The cell responds to the accumulation of nonfunctional proteins by the heat-induced upregulation of the heat shock proteins (HSPs), including both chaperones and proteases (2). The production of HSPs is regulated directly by alterations in the level, activity, and stability of the alternative sigma factor sigma-32 (3). The logic of the heat shock response is implemented through a hierarchy of feedback and feedforward controls that regulate both the amount of sigma-32 and its functionality. Upon a temperature upshift from 30°C to 42°C, sigma-32 levels rapidly increase during what is referred to as the induction phase. Subsequently, sigma-32 levels gradually decrease during the adaptation phase, reaching a new steady state level that is higher than the level prior to the temperature upshift.

Figure 1. The regulation architecture of the heat system in Escherichia coli. The regulation strategy includes a feedforward control loop that regulates the synthesis of sigma-32, and two feedback control loops that regulate the activity and stability of sigma-32. The architecture of the heat shock response regulatory network is not unlike that found in many engineering systems.
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Regulation Strategies: Feedforward and Feedback

We have developed a computational dynamic model that captures known aspects of the heat shock system (8). With the aid of this model, we discuss the logic of the heat shock response from a control theory perspective, drawing comparisons to synthetic engineering control systems. We begin by exploring the regulation of sigma-32 synthesis. At low temperatures, the sigma-32 messenger RNA (mRNA) has a secondary structure through base pairings, which has the effect of making translation of sigma-32 very inefficient. The increase of temperature leads to a fast melting of the secondary structure opening up the mRNA for a much more efficient translation of sigma-32 (4). This is akin to what control theorists refer to as feedforward control strategy. The heat shock system responds immediately to the disturbance (increased heat) by increasing the synthesis rate of the sigma factor. This direct response to the disturbance ensures a speedy heat shock response by not waiting until the effect of the increased temperature on the cellular proteins is sensed (i.e., no feedback is necessary). Feedforward strategies are very common in control engineering applications. One example is that of a driver on the freeway trying to maintain a constant distance with the car ahead. He or she would depress the brake pedal in response to seeing the brake light of the car ahead in anticipation of the imminent deceleration. This takes place before the driver is able to measure and respond to a decrease in the distance between the two vehicles (feedback control).

While the regulation of sigma-32 synthesis implements a feedforward strategy, the regulation of sigma-32 activity relies heavily on feedback. The feedback loop that results is worth explaining in some detail. At physiological temperatures, there are relatively few denatured proteins, a scenario that changes quickly upon temperature increase. As cellular proteins misfold, HSPs that act as chaperones (e.g., DnaK/DnaJ) bind to the unfolded proteins in an effort to refold them. As they do so, sigma-32 is free to bind RNA polymerase, enabling the complex to find the heat shock promoter site and to express more HSPs (3). When the folding state of the cell improves, this leads to an increase of free chaperones. These in turn bind sigma-32 molecules—whose hydrophobic residues make them appear as unfolded proteins—and in the process sequester sigma-32 away from RNA polymerase. The titration of the sigma-32 ensures that the expression of HSPs is reduced. This feedback loop just described acts by regulating the activity of sigma-32. We will discuss the advantages of this loop later in this article.

Another, more subtle feedback loop regulates the stability of sigma-32. FtsH, a membrane-bound protease, which is encoded on the heat shock regulon, degrades sigma-32. Studies suggest that rapid degradation of sigma-32 by proteases requires DnaK/DnaJ chaperones. For the first 5 min after temperature upshift sigma-32 is stabilized. The likely cause is that during this time, the chaperones are unavailable for the degradation reaction, because they are sequestered by unfolded proteins (5,6). This implements a degradation feedback loop. It should be mentioned that this regulation of stability through feedback as we just described is not necessary for a functioning heat shock response mechanism. Indeed, regulation of synthesis and activity alone would be sufficient to create a workable heat shock response mechanism. The strategy of regulating the stability through feedback (as opposed to constitutive degradation) is part of the overall regulation strategy of the heat shock response.

Regulation Analysis

We have used our computational dynamic model to analyze the various strategies of the heat shock system (7–9). The model itself consists of a set of 31 differential algebraic equations that describe the evolution of concentration of the various key players in the heat shock response, including the sigma-32 mRNA, sigma-32, DnaK/DnaK, FtsH, and folded and unfolded cellular proteins. The computational model reflects temperature effects on sigma-32 synthesis and on the unfolding rate of cellular proteins. The model simulations started before a temperature upshift and continued throughout a sudden increase in temperature and into the adaptation phase. Simulated time trajectories of sigma-32 and chaperones closely resemble the characteristic response of these proteins that is seen in wet experiments. Furthermore, the computer model captures the effect of various mutants, such as FtsH null, that have been measured experimentally. Using this model, we have explored the effects of several in silico mutants in an effort to better understand the role of the feedforward loop.
and the two feedback loops discussed earlier. We have also used other methods from control theory to analyze the overall system, including tools for sensitivity and robustness analysis, tools for model validation, and tools for studying the optimality of the system.

The result of our analysis allowed us to elucidate the underlying architecture of the system and to get a deeper understanding of the various strategies involved. By disabling the feedforward loop and each of the feedback loops, one loop at a time, we were able to understand their role in the overall response. The result of these numerical experiments is shown in Figure 2.

To summarize our findings, we have seen that the disturbance feedforward is responsible for generating a fast response to temperature increases. The system responds to increased temperature without feedforward, but at a significantly slower rate. On the other hand, the sequestration feedback loop that regulates the activity of the sigma-32 is responsible for the remarkable robustness that system has to variations in various parameters. It also makes the response more efficient by avoiding overexpression of HSPs beyond what is needed. The degradation feedback loop that is responsible for regulating stability was found to be largely responsible for the characteristic peak that is seen after the induction phase but before the adaptation phase (see Figure 2). Compared to constitutive degradation, regulated degradation further enhances the speed of the response, and can be shown to suppress fluctuation due to noise in a stochastic model of the system (8).

Motivated by the remarkable efficiency that we have observed, a feature that is reflected in the speed of the response and the efficient use of chaperones to achieve it, we have carried out optimality studies to explore the possible optimality of the heat shock system. Using tools from optimal control theory, we looked at a cost function that reflects the level of unfolded proteins as well as the amount of chaperones used to achieve such levels. This aggregate measure was then minimized through an optimal choice for model parameter values. Interestingly, the optimal value of this measure was very close to the value achieved by the wild-type model itself, suggesting that the wild-type model achieves optimal performance—at least with respect to the two criteria used.

Connection to Experiment Design

Aside from providing a deeper understanding of biological complexity, the modeling approach described in this article can aid in experiment design. For example, it can be shown that a dynamic computational model that does not include mechanisms of regulated stability cannot yield time trajectories that are consistent with experimental data, regardless of the simulated model parameter values. Thus, a model with no regulated degradation does not describe all the essential pieces of the heat shock response and strongly suggests that further experiments are needed to look for additional players or interactions. As another example, it has been long known that the induction phase in the heat shock system is attributed to the partial melting of the secondary structure of the sigma-32 mRNA, which implements a built-in RNA thermosensor that induces a sudden and sharp increase in the cellular level of sigma upon heat shock. This is the feedforward loop discussed earlier. During the adaptation phase, the sigma-32 level declines until it reaches a new steady state value. This decline was hypothesized to be the result of a posttranscriptional repression of sigma-32 synthesis (10). The search for such a mechanism came up empty, however. In silico simulations of the computational model would have easily concluded that the known dynamics of the heat system are in fact sufficient to reproduce the observed adaptation without the need for a translation shut-off. These results agree with the experimental results of Reference 11, which prove conclusively the absence of a sigma-32 translation shut-off mechanism.

In conclusion, we have used methods from control theory to study the heat shock response system. We have shown that the architecture of the heat shock response is a necessary outcome of robustness and tight performance requirements, such as speed. We have also found that despite their vastly different substrates, biological regulatory mechanisms and their synthetic counterparts used in engineering share many similarities, as they are both subject to the same fundamental constraints that govern all regulatory mechanisms. The architectures of both cellular and engineered regulatory networks exploit sensors to measure disturbances and regulated variables of interest and use these measured signals to induce action that regulates the outputs of interest through a network of feedback and feedforward loops. Notions used in the study of engineering control systems such as optimality, nonlinearity, robustness, isolation, modularity, and feedback are invaluable for understanding biological complexity, including the reverse engineering of biological systems with the goal of understanding how they achieve robust function.

References


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