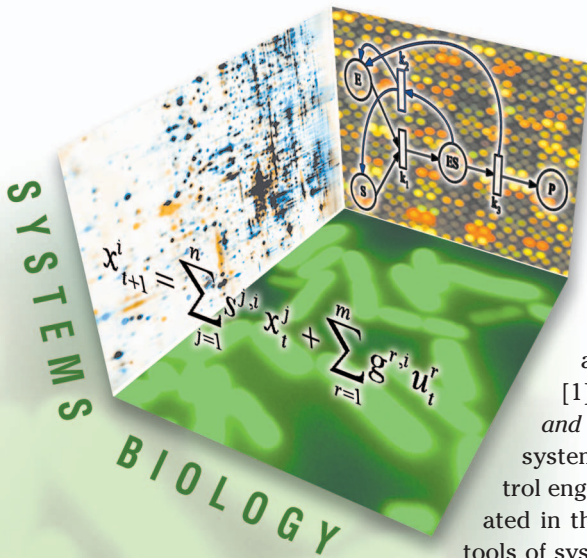


By Mustafa Khammash and Hana El-Samad

Systems Biology: From Physiology to Gene Regulation

Engineering approaches to the
study of biological sciences



Feedback seems to be the strategy of choice in biological systems. “Regulation” and “feedback” are terms used by life scientists to describe and explain various biological phenomena at the physiological and molecular levels. As early as 1948, homeostasis, or the ability of biological mechanisms to restore their equilibrium in the presence of disturbances (also known as the *fixite du milieu interieur*), was attributed by Wiener to feedback control present in these mechanisms [1]. In his book *Cybernetics, or Control and Communication in the Animal and the Machine*, Wiener established the necessity of interaction between systems theory and the biological sciences. Physiologists [2], [3] and control engineers [4], [5] shared his perspective, and research projects were initiated in the 1960s and 1970s to study biological regulatory systems using the tools of systems analysis. Physiologic systems of interest include the respiratory and cardiovascular systems; thermoregulation [4]; endocrine regulation; gastrointestinal secretions; water exchange control [3], [6]; systemic blood pressure; renal plasma clearance; blood glucose control; and pupillary reactions [7]. Interest also arose in the modeling of cellular behavior and gene regulatory networks. Researchers have studied mathematical [8] and mechanistic schemes for gene regulation [9], yielding important insight into the operation of these systems.

Currently, there is a revival of interest in mathematical methods as necessary tools for understanding biological organization. Many factors motivate this renewed interest. First, advances in molecular biology over the past decade have made it possible to experimentally probe cause-and-effect relationships between microscopic processes initiated by individual molecules within a cell and their macroscopic phenotypic effects on cells and organisms. These studies provide detailed snapshots of the underlying networks, circuits, and pathways responsible for the basic functionality and robustness of

biological systems and create new and exciting opportunities for the development of quantitative and predictive models. Second, the constructed models can be numerically simulated using algorithms that can address their complexity and exploit their structure for more efficient solutions. Finally, modelers and experimentalists motivated by success stories [10] are trying to bridge the gap between disciplines, establishing various collaborations where iteration between modeling and experiments is rapidly advancing knowledge in both fields. Successful engineering [11] and reverse engineering [12] of biological systems has proven to be a feasible task, leading to the development of new modeling techniques and mathematical theories (see [13] and the other articles in this special section).

In this article, we review models for two biological systems. First, we present a physiologically critical control mechanism that regulates the calcium level in mammals. We then describe the heat-shock (HS) response, an evolutionary conserved gene regulatory network that ensures the survival of various organisms at different temperatures. In both cases, we offer a control engineering perspective and demonstrate how this perspective deepens our understanding of the structure and functionality of these systems. We refer the interested reader to [14]–[16] for a detailed exposition of model development and equations.

Plasma Calcium Homeostasis in Mammals

Calcium homeostasis is necessary for survival in mammals. At the physiological level, calcium salts maintain the integrity of the skeleton. Calcium cations are also essential components for cellular function. For example, intracellular calcium cations are information conveyors from the surface to the interior of the cell, and extracellular calcium cations are crucial for blood clotting, neuromuscular excitability, and various hormonal secretion mechanisms [17]–[19]. This physiological role of calcium necessitates that its concentration be precisely monitored and controlled and that any deviation from the physiologically necessary setpoint (0.085–0.105 g/l in humans [19] and 0.08–0.1 g/l in dairy cows [20]) be tightly regulated. Such regulation of the plasma calcium concentrations is achieved by modulating calcium influx/outflux to the blood from bone, the kidneys, and the intestines. These fluxes are under hormonal control, and their magnitude changes according to the deviation of the calcium concentration from its setpoint. By denoting the total rate of calcium introduced into the plasma as $V_T(t)$ (g/day) and the total calcium clearance from the plasma as $V_{cl}(t)$ (g/day), the net rate of change of plasma concentration is given by

$$\frac{d[Ca]_p}{dt} = \frac{1}{\text{vol}} (V_T(t) - V_{cl}(t)), \quad (1)$$

where $[Ca]_p$ (g/l) denotes the concentration of plasma calcium, and vol denotes plasma volume. In mammals, a dis-

placement of V_{cl} from its nominal value occurs due to variation in the dietary calcium concentration or the calcium demand to meet milk production and fetal growth needs. The relation in (1) represents the calcium “plant” to be controlled. The overall calcium homeostatic closed-loop system is depicted in Figure 1(a). Our goal is to elucidate the control scheme used in this system. This investigation is essential for understanding calcium-related diseases that are likely to arise from the failure of one or more of the components of the control mechanism itself. In this endeavor, we shall focus on calcium homeostasis in dairy cows, keeping in mind that other mammalian calcium control schemes are of a similar nature.

The standard model for the calcium homeostatic controller given in [21] is based on experimental data collected at the onset of lactation in dairy cows, a time that coincides with calving (also known as “parturition”). Right before parturition, a sudden and large increase in the lactational need for calcium is observed as a step disturbance in V_{cl} from 20 to 70 g/day [see Figure 1(a)]. The control law in [21] is empirically calculated as a proportionality constant between V_T and the tracking error $e(t)$; that is,

$$V_T = K_p e(t) \simeq 1770 e(t) \text{ (g/day)},$$

which represents proportional feedback. Therefore, the transfer function between the tracking error $e(t)$ and the disturbance V_{cl} is

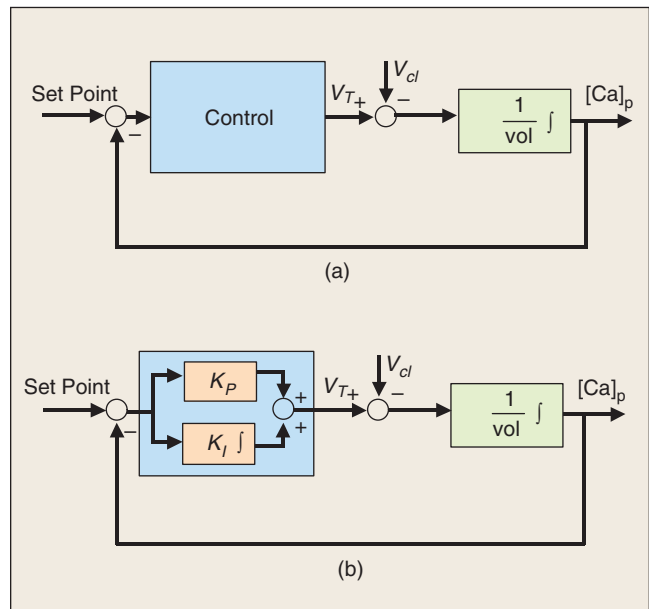


Figure 1. (a) Block diagram of the calcium regulatory system. The calcium level, which is the output of interest, is fed back to a controller that adjusts the calcium flux. (b) The proposed proportional-plus-integral (PI) control law for the calcium regulatory system. PI control is necessary to perfectly reject step changes in V_{cl} .

$$\frac{e(s)}{V_{cl}(s)} = \frac{\overline{V_{cl}}}{s + \frac{K_p}{\text{vol}}}, \quad (2)$$

where $\overline{V_{cl}}$ is the strength of the disturbance. For a unit step disturbance, it follows from the final value theorem applied that the steady-state tracking error is given by

$$e(\infty) = \frac{\overline{V_{cl}}}{K_p}.$$

Since $e(\infty)$ is nonzero, the plasma concentration fails to return to its setpoint value in response to a step change in V_{cl} , and a steady-state error persists. The step response is shown in Figure 2(a).

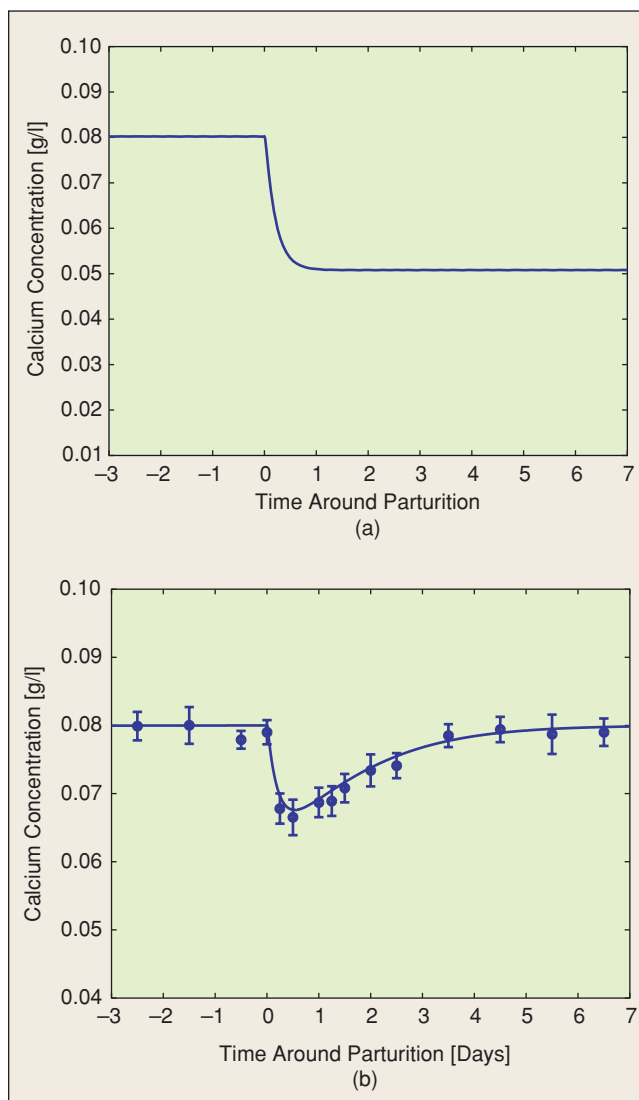


Figure 2. (a) Calcium level for the proportional control model. (b) Experimental data and simulation results for the PI control system. The points on the plots correspond to the average plasma calcium concentration for 18 calving cows. Results are presented as means \pm standard error. The calcium disturbance occurs at time zero.

Therefore, proportional control cannot explain the observed plasma calcium concentration time histories. Indeed, calcium data strongly indicate that most animals adapt to relatively large calcium demand and that the plasma calcium concentration returns to normal after a transient period of reduced concentration. This effect has been observed in calving cows, where the calcium levels mostly recovered to their predisturbance value despite a four- to five-fold increase in the rate of calcium clearance from the plasma [22], [23] [see Figure 2(b)]. This perfect adaptation implies a zero steady-state tracking error to a step disturbance, which cannot be explained by proportional feedback. Furthermore, the second-order transient response to a step disturbance characteristic of a sudden increase in calcium demand [Figure 2(b)] cannot result from a first-order system, where proportional feedback alone is in place.

The Necessity of Integral Feedback

The internal model theorem [24] dictates that integral action must be present to achieve zero steady-state tracking error. We thus assert that achieving V_{cl} step rejection requires that an integral term be included in the feedback model for V_T . Hence, the proposed proportional-integral (PI) control term is

$$V_T = K_p e + K_I \int e,$$

where K_p and K_I are constants and e is the calcium regulation error. The block diagram of the system is shown in Figure 1(b).

Obviously, the most immediate consequence of this PI control law is that perfect adaptation observed in experimental data is both a structural and robust property of the model. Another consequence is the fact that the calcium output has the same transient response characteristics as experimental calcium data. Figure 2(b) illustrates the simulated response of the system model plotted against experimental data. The simulation parameters K_p and K_I are calculated to minimize the mean square error between the model time profile and experimental data taken from 20 calving cows. The data points used for model verification are obtained from an independent set of 18 calving cows.

Origins of Integral Action (or How Cows Integrate)

In engineering systems, PI control is usually implemented through a variety of well-defined schemes and devices, such as operational amplifier circuits. In the setting of calcium homeostasis, it is interesting to look for a physiological basis for PI control. Since it has been established that the calcium level is hormonally controlled, we investigate possible schemes of hormonal interactions to explain our hypothesized PI control. We start by considering whether

PI feedback can be realized with a single hormone, say, hormone A. Suppose that the total calcium input V_T into the plasma is proportional to the concentration of Hormone A (denoted by [Hormone A]); that is,

$$V_T \propto [\text{Hormone A}].$$

Then PI feedback can be explained, provided

$$\frac{d}{dt}[\text{Hormone A}] \propto \left(\text{error} + K \frac{d}{dt} \text{error} \right). \quad (3)$$

There are two reasons why (3) is not a likely scenario, however. First, (3) requires that the production rate of Hormone A be dependent on two separate processes: one proportional to the error and the other proportional to the error derivative. Second, (3) requires derivatives of the error signal, which is difficult to achieve due to noise at the input of the differentiator.

If, however, *two* hormones—A and B—are allowed, a more elegant and likely possibility emerges. Suppose that the following conditions are satisfied:

- The concentration of Hormone A is proportional to the error; that is,

$$[\text{Hormone A}] \propto \text{error}.$$

- The production rate of Hormone B is proportional to the concentration of Hormone A; that is,

$$\frac{d}{dt}[\text{Hormone B}] \propto [\text{Hormone A}].$$

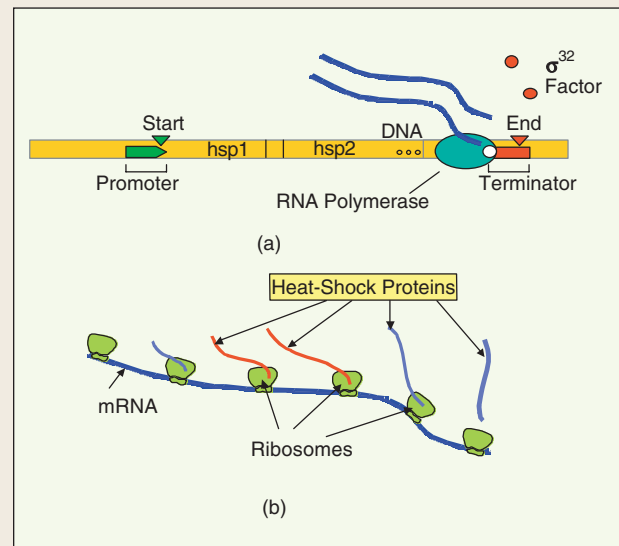
- $V_T = V_A + V_B$, where $V_A \propto [\text{Hormone A}]$ and $V_B \propto [\text{Hormone B}]$.

In this case, it is easy to see that the proportional component of PI control (3) is given by V_A , while the integral component is given by V_B .

Transcription and Translation

The synthesis of heat-shock proteins (HSPs) involves the following sequence of events. The enzyme RNA polymerase (RNAP) bound to the regulatory sigma factor σ^{32} recognizes a sequence in the DNA referred to as *HS promoter*, which lies just before the sequence encoding the HS genes [see (a) below]. Whereas the role of RNAP is to transcribe genes, the main role of σ^{32} is to recognize the HS promoter sequence to signal to RNAP to initiate the transcription of heat-shock (HS) genes. The transcription process consists of creating a messenger RNA molecule that carries the information encoded by the HS genes. RNAP thus acts as a “reading head,” transcribing DNA sequences into mRNA. Once a few nucleotides on the DNA have been transcribed, the σ^{32} molecule is released back into the cell, while RNAP continues transcribing the HS genes until it recognizes a terminator sequence. At this point, the mRNA is complete, and RNAP disengages from the DNA. The HS genes encode predominantly molecular chaperones (such as DnaK, DnaJ, GroEL, GrpE), as well as proteases (such as Lon, FtsH). Chaperones are molecules responsible for refolding denatured proteins, while proteases degrade unfolded proteins. Transcribed into mRNA, ribosomes translate the message on mRNA, producing HS proteins (both chaperones and proteases) [see (b) below]. This process of translation consists of sequentially assembling amino acids in an order that corresponds to the mRNA sequence, with each set of three

nucleotides corresponding to a single unique amino acid. This combined process of gene transcription and mRNA translation constitutes gene expression and is often referred to as the central dogma of molecular biology.



(a) The transcription process: the factor σ^{32} identifies the promoter site on the DNA, thereby signalling the enzyme RNA polymerase to initiate transcribing the DNA gene sequence into messenger RNA. (b) The translation process: the mRNA is translated by ribosomes into the linear sequences of amino acids that make up the encoded proteins. Each amino acid is represented by three nucleotides.

In the two-hormone scheme, the concentration of Hormone A provides a measure of the error, while the concentration of Hormone B provides a measure of the integral of the error. Therefore, only the concentration of Hormone A, rather than its rate of change, is needed to determine the production rate of Hormone B, much like a catalyst concentration would determine the rate of a chemical reaction. We next investigate whether the two-hormone hypothesis is supported by known physiology.

Endocrinology of Calcium Homeostasis

Calcium homeostasis is achieved through the inflow of calcium from bone, the kidneys, and the intestines under the control of two major hormones: parathyroid hormone (PTH) and an important metabolite of vitamin D, 1,25-Dihydroxycholecalciferol (1,25-DHCC) [19]. PTH is secreted by the parathyroid glands in response to a decrease in the calcium plasma concentration from its setpoint. Experiments have shown that this production can be accurately represented by a linear function of the deviation from the setpoint [25]. PTH acts mainly on bone and the kidneys. In response to the increase in PTH concentration, a process known as “osteocytic osteolysis” occurs, in which PTH causes the removal of bone salts from the bone matrix by lacunar osteocytes. This process occurs within minutes and proceeds without actual resorption of bone matrix [17]. Most short-term needs are met through osteocytic osteolysis. If high concentrations of PTH persist, a delayed response of hours to days, known as “osteoclastic bone resorption,” takes place due to the activation of the bone osteoclasts. This process involves resorbing the bone matrix and allows the response to PTH to continue beyond what can be handled by osteocytic osteolysis. Thus, the need for maintaining plasma calcium concentrations is deemed more important than maintaining the integrity of bone. The effect of PTH on the kidneys is to increase tubular reabsorption of calcium, thus reducing calcium loss through urine. Therefore, the impact of PTH is to increase immediate calcium transfer into the blood plasma.

On the other hand, the main role of 1,25-DHCC is to stimulate intestinal calcium absorption through increasing formation of a calcium-binding protein in the intestinal epithelial cell [18]. In fact, 1,25-DHCC is considered to be the most potent stimulator of calcium absorption from the intestine. 1,25-DHCC is produced from cholecalciferol, a biologically inactive form of vitamin D, after it undergoes several hydroxylation steps in the liver and kidneys [17]–[20]. The last hydroxylation step in the kidney takes place only under stimulation by PTH. Calcitonin, the third hormone involved in calcium homeostasis, has relatively little relevance during hypocalcemia, and, therefore, is not considered. In fact, calcitonin is not secreted until plasma calcium levels exceed 0.095 g/l. Above this calcium level, plasma calcitonin is directly proportional to plasma calci-

um [25]. To describe these interactions mathematically, we define V_{bone} to be the rate at which calcium is transported into the plasma from bone. The hormone PTH is the main stimulant of bone resorption, and, hence, the rate of calcium release from the bone is, to a first-order approximation, proportional to its plasma concentration. Therefore, for some $\alpha_b > 0$,

$$V_{\text{bone}} = \alpha_b[\text{PTH}].$$

Moreover, the PTH plasma concentration is known to be proportional to the calcium error [26]; that is,

$$[\text{PTH}] = \alpha_e e.$$

Therefore

$$V_{\text{bone}} = K_p e, \quad (4)$$

where $K_p = \alpha_b \alpha_e$.

Similarly, since intestinal absorption is stimulated by 1,25-DHCC, we approximate its rate by a linear function of the 1,25-DHCC concentration; that is,

$$V_{\text{intestine}} = \alpha_i [1,25\text{-DHCC}] \quad (5)$$

for some $\alpha_i > 0$. As previously mentioned, the last hydroxylation step of cholecalciferol in the kidney occurs under PTH stimulation. Thus, assuming a large buffered pool of cholecalciferol and considering the large half-life of 1,25-DHCC [27], the rate of production of the biologically active 1,25-DHCC will be directly proportional to the PTH concentration. Thus

$$\frac{d}{dt}[1,25\text{-DHCC}] = \alpha_p [\text{PTH}],$$

which implies that

$$[1,25\text{-DHCC}] = \alpha_p \int_0^t [\text{PTH}] d\tau. \quad (6)$$

Therefore, (5) and (6) together yield

$$V_{\text{intestine}} = \alpha_p \alpha_i \int_0^t [\text{PTH}] d\tau. \quad (7)$$

Replacing [PTH] in (7) by its expression in (4), we obtain

$$V_{\text{intestine}} = K_I \int_0^t e d\tau, \quad (8)$$

where $K_I := \alpha_i \alpha_p \alpha_e$. If we associate Hormone A with PTH and Hormone B with 1,25-DHCC, (4) and (8) together define a PI controller that is exactly implemented through the hormonal interactions depicted in the second case of our proposed scheme.

A Deeper Understanding of Calcium Homeostasis Through Control Theory

Although mathematically simple, the calcium homeostasis model carries key concepts that form the core of biological modeling. The calcium model demonstrates that an approach based on observing the dynamics of a biological system versus cause-and-effect static relationships is essential for establishing constraints on its structural implementation and is necessary for the presence or absence of key components in this implementation. Indeed, the profound implications of the robust adaptation of calcium level to disturbances on the structure of the underlying homeostatic mechanism have gone unrecognized in the literature, as evidenced by the proportional feedback homeostasis model used in [21]. When one considers this indicative behavior and resorts to powerful concepts such as the internal model principle, however, it becomes apparent that the necessity of integral feedback is inescapable. Once this integral feedback is in place, one can go back and verify that a model containing such mechanism exhibits transient characteristics similar to those seen in actual data. The most important implication of this approach does not lie solely in producing a simple dynamical model that agrees well with the actual data. Rather, it lies in the severe structural constraints that it imposes on the underlying homeostatic mechanism. Such constraints explain the role of PTH and 1,25-DHCC in homeostasis and the nature of the interaction between these two hormones as a direct result of the requirements of integral control—so much so that the mere existence of two hormones responsible for calcium homeostasis, along with their function and the nature of their mutual interaction, can be hypothesized based on the requirements of integral feedback control alone, without prior knowledge of the endocrinology of calcium homeostasis. The significance of this fact can be appreciated when one considers that 1,25-DHCC and its role in calcium homeostasis was discovered as late as the 1970s (as opposed to the role of PTH, which had been discovered much earlier). Furthermore, even when one takes for granted the presence of two hormones responsible for calcium homeostasis during hypocalcemia, in the absence of explanations that rely on integral feedback the only explanation for the need for two hormones as opposed to a single hormone is redundancy. Based on the arguments put forth in our exposition, this explanation must be abandoned.

Understanding the exact functional roles of mechanisms underlying biological homeostasis is essential for identifying the causes of their occasional breakdown and disease states. This understanding can be achieved by eliminating possibilities that are not consistent with the kind of dynamics producible by such mechanisms. For example, in the case of calcium homeostasis, one can use the PI model augmented by known nonlinear effects, such as saturation in

the bone response to PTH and reduction in gut motility during severe hypocalcemia to study milk fever, a disease attributed to the breakdown of the calcium homeostatic mechanism. The use of the calcium dynamic model suggests that a reduction in bone responsiveness to PTH is an important factor leading to milk fever. However, this reduced bone responsiveness must be accompanied by a reduction in gut motility, indicating that neither effect alone can be responsible for milk fever. This kind of information can be essential for suggesting further experiments or courses for the treatment of milk fever, all the while providing a head start for exploring the causes of human diseases such as osteoporosis.

Molecular Gene Networks: The Bacterial HS Response

Organisms are subject to a plethora of environmental and metabolic stress conditions, including environmental factors such as an increase in the ambient temperature, chemical stresses involving metabolically harmful substances, and viral infection. These sources of stress have a detrimental effect on the cell since they often cause partial or complete protein unfolding and denaturation. Since a protein's three-dimensional folded structure is essential for its proper function, unfolded or misfolded proteins present in large numbers disrupt normal cellular functions and, if not combated, result in cell death. Consequently, regulatory systems have evolved to detect the damage associated with stressors and to initiate a response that increases the resistance of cells to damage and aids in its repair.

Among the most important of these protective systems is the HS response [28]. The HS response consists of elaborate mechanisms for detecting the presence of heat or other stressor-related protein damage [29] and for initiating a response through the synthesis of new heat-shock proteins (HSPs) whose main function is to refold denatured cellular proteins. Indeed, cells have evolved cellular mechanisms for regulating the expression of specific genes that encode HSPs, thereby enabling the rapid synthesis of HSP in amounts that do not impose a heavy metabolic burden on the cell. Understanding the HS response and categorizing its functional blocks creates insight into the organization of gene networks and the way various control strategies act to regulate these networks to achieve robust and efficient cellular function. A well-studied HS response is that of the bacterium *E. coli*, which serves as an ideal case study for understanding complex molecular gene networks and their regulation.

The HS Response in *E. coli*

In *E. coli*, the HS response is implemented through an intricate architecture of feedback loops centered around the HS factor, a molecule referred to as σ^{32} , which regulates the transcription of the HS proteins under normal and

stress conditions. We describe this process in some detail to explain the role of σ^{32} .

The enzyme RNA polymerase (RNAP), bound to the regulatory sigma factor σ^{32} , recognizes the HS genes that encode predominantly molecular chaperones such as DnaK, DnaJ, GroEL, and GrpE, as well as proteases such

Understanding the exact functional roles of mechanisms underlying biological homeostasis is essential for identifying the causes of their occasional breakdown and disease states.

as Lon and FtsH. Chaperones are responsible for refolding denatured proteins, while proteases degrade unfolded proteins. Therefore, the HS factor σ^{32} plays an essential role in initiating the HS gene expression. It is not surprising that the regulation of the HS response is achieved through the tight regulation of σ^{32} . Interestingly, the synthesis, activity, and stability (time before degradation) of σ^{32} are all regulated. Such regulation is realized through feedback and feedforward loops that incorporate temperature data and the folding state of proteins in the cell. The mechanism of this regulation at the molecular level is quite elegant, and we describe it in more detail in the following section.

Feedforward and Feedback Mechanisms at the Molecular Level

At physiological temperatures (30 to 37 °C) there is little σ^{32} present and, hence, little transcription of the HS genes. When *E. coli* are exposed to high temperatures, σ^{32} rapidly accumulates, allowing increased transcription of the HS genes, and then declines to a new steady-state

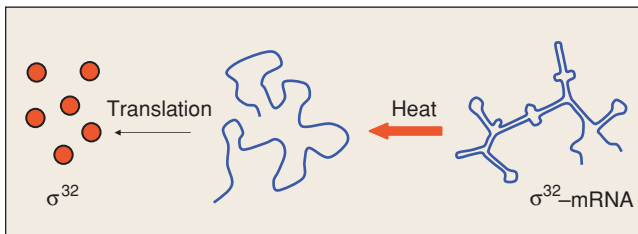


Figure 3. Translational control of σ^{32} synthesis. At low temperatures, ribosomes translate the σ^{32} -mRNA inefficiently due to the secondary structure locked by base pairings. At higher temperatures, the melting of these base pairings allows a more efficient translation. This mechanism embodies feedforward control.

level characteristic of the higher temperature. An elegant mechanism that senses temperature and immediately reacts to its effect is implemented as follows in the bacterial HS response. At low temperatures, the translation start site of σ^{32} is occluded by base pairing with other regions of the σ^{32} mRNA. Upon temperature upshift this base pairing is destabilized, resulting in a “melting” of the secondary structure of σ^{32} , which enhances ribosome entry, therefore increasing the translation efficiency. Indeed, the translation rate of the mRNA encoding σ^{32} increases immediately upon temperature increase [30]. Hence, a sudden increase in temperature, sensed through this mechanism, results in a burst of σ^{32} and a corresponding increase in the number of HSPs. This mechanism implements a

control scheme similar to a feedforward control loop 3 (see Figure 3). This mechanism renders the production of HSPs temperature dependent. We now describe two feedback mechanisms that regulate the activity and degradation of σ^{32} .

The chaperone DnaK and its cochaperone DnaJ perform protein folding. At the same time, these proteins can bind to σ^{32} , therefore limiting the ability of σ^{32} to bind to RNAP. Raising the temperature increases the cellular levels of unfolded proteins that then titrate DnaK/J away from σ^{32} , allowing σ^{32} to bind to RNAP and increasing the transcription of DnaK/J and other chaperones. Together, increased translation and decreased degradation lead to a transient 15- to 20-fold increase in the amount of σ^{32} at the peak of the HS response. The accumulation of high levels of HS proteins leads to the efficient refolding of the denatured proteins, thereby decreasing the pool of unfolded protein, freeing up DnaK/J to sequester this protein from RNAP. This process implements a sequestration feedback loop (see Figure 4). The activity of σ^{32} is regulated through a feedback loop that involves competition between σ^{32} and unfolded proteins for binding with the free DnaK/J chaperone pool.

During steady-state growth, σ^{32} is rapidly degraded ($t_{1/2} = 1$ min) but is stabilized for the first five minutes after temperature upshift. The chaperone DnaK and its cochaperone DnaJ are required for the rapid degradation of σ^{32} by the HS protease FtsH. RNAP-bound σ^{32} is protected from this degradation. Furthermore, FtsH, a product of the HSP expression, experiences a synthesis rate that is tied to the transcription/translation rate of DnaK/J. Therefore, as protein unfolding occurs, σ^{32} is stabilized by the relief of its sequestration from DnaK. However, as more proteins are refolded and as the number of FtsH increases, there is a decrease in the concentration of σ^{32} to a new steady-state concentration dictated by the balance

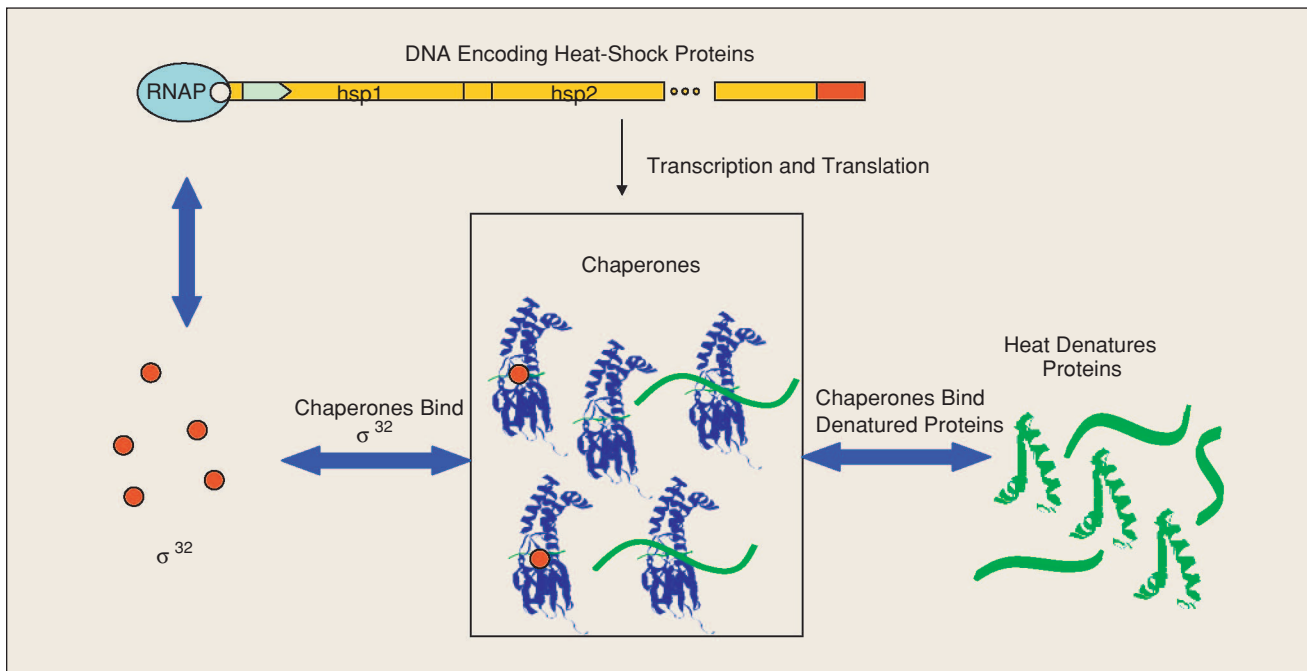


Figure 4. Regulation of σ^{32} activity. σ^{32} is sequestered by the chaperones, therefore limiting its ability to bind to RNA polymerase. This sequestration is relieved by the presence of a large number of unfolded proteins.

between the temperature-dependent translation of the σ^{32} mRNA and the level of σ^{32} activity modulated by the HSP chaperones and proteases acting in negative feedback. In this way, the FtsH-mediated degradation of σ^{32} is feedback regulated. We refer to this process as the FtsH degradation feedback loop. A biological block diagram of the HS response that shows the various regulation mechanisms is shown in Figure 5.

Modeling and Analysis of Control Strategies

A deterministic mathematical model for the heat stress response in *E. coli* developed in [14] and [15] uses first-order mass-action kinetics and describes the synthesis of new proteins as well as the association and dissociation activity of molecules. The dynamics of the different components involved are described by differential rate equations, and the full model takes the form of a set of 31 differential-algebraic equations with 27 kinetic parameters. The model is simulated using the specialized software DASSL [31]. This detailed model is validated against experimental data and is shown to reproduce the qualitative and quantitative behavior of the wild-type HS response and its mutants. A reduced-order model derived in [32] captures the essential features of the HS response.

Designing a minimal HS system is, in principle, fairly simple. One approach requires transcriptional/translational machinery for the HS genes that respond to an increase in temperature. The products of gene expression are chaperones that refold denatured proteins. This scheme can be

achieved by simple components acting in an open-loop fashion and does not necessarily require the level of complexity that is seen in the HS system. What lies behind this complexity? One might be tempted to attribute this complexity to evolutionary accidents or redundancy. As in advanced engineering systems, however, it can be shown that much of the system complexity is due to the presence

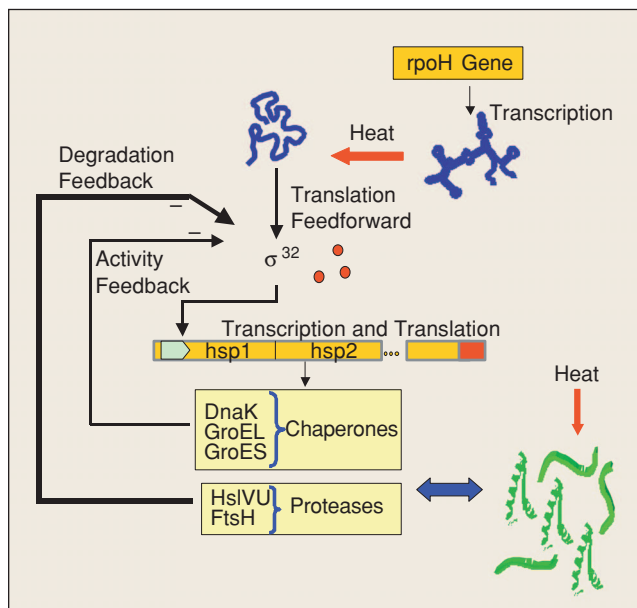


Figure 5. Biological block diagram of the HS response. The figure shows two feedback loops and a feedforward loop characteristic of the regulatory structure of the HS system.

of hierarchies of feedback loops that are responsible for achieving robustness, performance, noise rejection, and resource constraints. Our approach to investigating this complexity is to start with a minimal system that achieves basic functionality and then add successive layers of regulation, demonstrating how each layer improves the performance of the overall system.

The complex nature of biological systems makes an integrative systems approach essential.

We start by considering the simplest functional design that uses σ^{32} to produce HSPs. This hypothetical design, shown in Figure 6(a), consists of an open-loop design strategy where the number of σ^{32} molecules dictates the level of HSPs in the cell. Hence, any number of σ^{32} and HSPs is achievable by carefully tuning the synthesis rates of these proteins. As one might expect, however, a single set of these parameters cannot yield satisfactory performance for all temperatures since the production of HSPs must increase along with temperature in order to achieve the protein-folding task.

The translational regulation mechanism of σ^{32} provides a means for sensing temperature and for immediately reacting to its effect. This mechanism renders the production of HSPs temperature dependent. This dependence is a considerable improvement over the constant σ^{32} production rate scenario but requires the added complexity associated

with implementing the temperature-dependent σ^{32} translation-modulating mechanism. This sensing mechanism and the use of the temperature information to affect the production of HSPs independently of the folding state of the cellular proteins represents a feedforward control strategy.

Although open-loop design with feedforward functionality is appealingly simple, this strategy suffers from severe shortcomings—the most critical being the lack of robustness. In fact, open-loop designs are adequate only when the external cellular environment is constant and the system components are certain. Outside this ideal world, feedback control is necessary to enable cellular function despite imprecise components and the ever-changing cellular environment. Indeed, in this hypothetical open-loop design of the HS response, the slightest change in the transcription and translation rates results in a corresponding change in the number of HSPs produced. This sensitivity to parametric uncertainty is one of the key reasons that feedback control systems are superior to open-loop systems. This property of feedback to transform an otherwise wildly varying and unpredictable open-loop system into one where signals are maintained within tight tolerances, despite component uncertainty, has led to the pervasive and successful use of feedback control systems in numerous engineering disciplines. As in man-made engineering systems, the hypersensitivity to parameter variations in the HS response seen in the open-loop design is circumvented through the use of feedback. The elegant implementation makes use of the chaperones that sequester free σ^{32} , thus modulating the pool of σ^{32} available for RNAP binding, thereby suppressing the production of chaperones through this feedback path.

Up to this point, the repair mechanism following temperature-induced damage has been implemented through a feedforward term that anticipates the damage and immediately reacts to it, in addition to a sequestration feedback loop that endows the system with robustness against parametric variations. In these hypothetical models, the production and activity of σ^{32} are regulated, while degradation is assumed to take place at a constant rate. If this degradation is also regulated based on the protein-folding state of the cell, thereby adding an extra feedback loop to the system [see Figure 6(b)], the result is predictably a further enhanced robustness.

Robustness apart, feedback loops also have a dramatic effect on the transient dynamics of the HS system. Notably, these loops contribute to the reduction of the delay in the folding response to the heat disturbance, as shown in Figure 7.

One feature of primary importance in cellular processes is the ability of cells to attenuate undesirable noise. Analyzing this feature requires that the cellular compo-

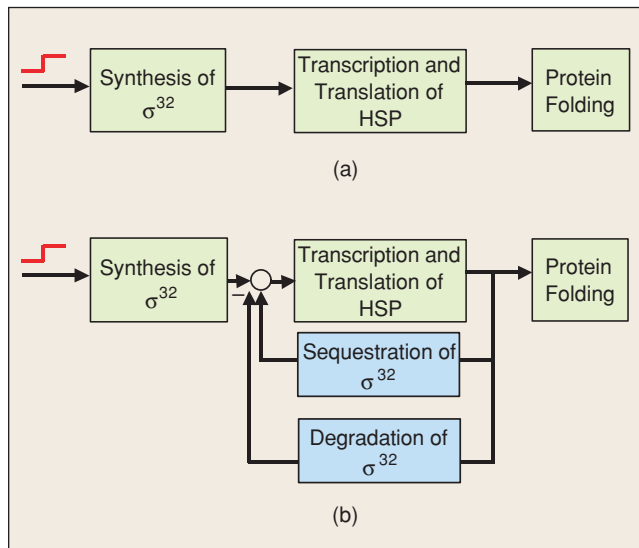


Figure 6. Hypothetical design models for the HS response: (a) shows the open-loop design with feedforward control that achieves the basic functionality of protein folding; (b) shows the closed-loop design with sequestration loop and degradation loop.

nents be modeled as random or stochastic chemical processes. The nature of stochasticity in these processes stems from various sources of uncertainty inside the cell and has been termed “intrinsic noise,” to differentiate it from the extrinsic noise that results from the environment [33], [34]. Various structures and strategies have been identified as leading to intrinsic noise rejection or exploitation mechanisms. For example, feedback has been observed experimentally to attenuate intrinsic cellular noise [35]. A stochastic description of the HS system was used in [36] to study the effect of different control strategies on noise rejection. In general, a stochastic representation of a well-stirred, constant-volume system of chemical reactions uses the chemical master equation (CME) formalism, which describes the evolution of probabilities rather than concentrations of molecular species [37]. Although the CME is usually not solvable, Monte Carlo-type algorithms, such as the Gillespie stochastic simulation algorithm, are used to generate sample paths of the process based on the statistical properties given by the CME [38]. For the HS response system, we demonstrate in [36] the role of the degradation feedback loop in noise rejection. Indeed, Gillespie’s algorithm may be used to stochastically simulate the HS system in the presence and absence of this loop. Figure 8 shows two sample paths, one without the degradation loop and the other with the loop in place. It is apparent that this feedback loop is instrumental in reducing the stochastic fluctuations around the steady state of the HSPs.

Opportunities and Challenges

The design principles that engineered systems share with natural systems are continuously being explored [39]. The realization that these types of systems share a large set of common features motivates mainstream attention in the mathematical study of biological systems. Using experience and insight from engineering offers the promise of generating more powerful tools for attacking biological complexity. At the same time, there are unique features where biological systems diverge from synthetic ones. The challenge of successful systems biology lies partly in the characterization of these features, followed by the successful extension of engineering and mathematical tools and the creation of new tools and theories to accommodate and exploit these unique features. We next outline research directions and opportunities along these lines.

Connecting Experimental Data to Models

In engineering sciences, system identification ideas are usually adopted to connect data to postulated models and their dynamics. In life sciences, however, static cause-and-effect relationships are often used to devise models for biological processes. Negative or positive relationships

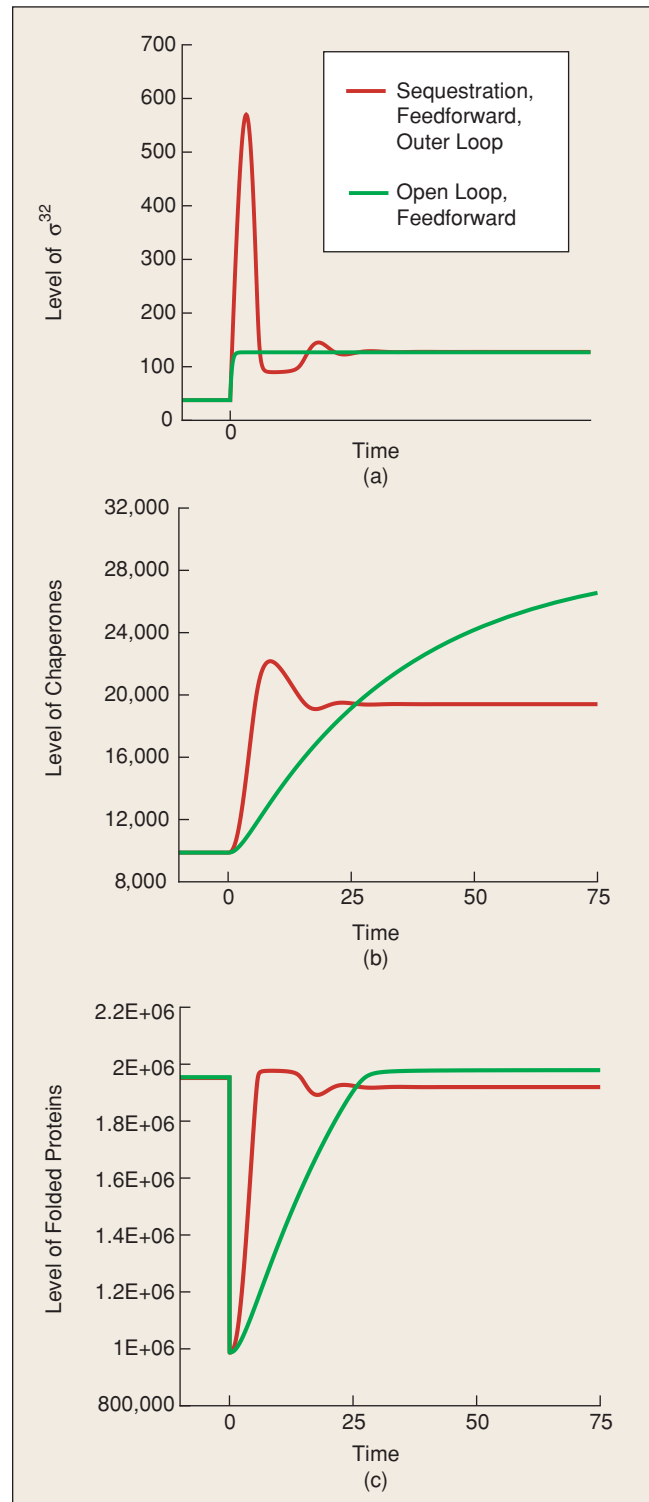


Figure 7. The levels of (a) σ^{32} , (b) DnaK, and (c) unfolded proteins for a design with added sequestration and degradation. The parameter values are chosen to have the same steady-state concentrations of σ^{32} and chaperones in open loop (green) and closed loop with sequestration and degradation (red) at low temperature. The performance is assessed at high temperature with HS at time zero.

between quantities are postulated based on the direct observed effect of one on the other. Missing in this picture is the idea of dynamics and whether the experimentally uncovered interactions are necessary and sufficient to reproduce the observed experimental data.

Reductionist biology has been successful in discovering the various basic components of a system and uncovering interactions among these components. When constructing a systems-level understanding, dynamic models are essential. One question is that of sufficiency: Are the known components and their interactions, as captured by dynamic models, sufficient to robustly reproduce the observed experimental data? Another question is that of necessity: Do the experimental data impose constraints on the biological system that necessitate the presence of certain components or specific interactions that are as yet unaccounted for in the model?

We have shown that ideas from control theory can be used to address some of these issues; for example, through the derivation of necessary conditions for the structure of a biological system and the type of control used to achieve homeostasis. We demonstrate how these conditions are used to eliminate some hypotheses and favor others and how biology can be used to gain more confidence in the favored hypotheses. In the calcium system, we prove the necessity of integral control to explain perfect adaptation. This perfect adaptation has also been observed in other biological systems, such as the chemotactic system in bacteria [40] and integral feedback postu-

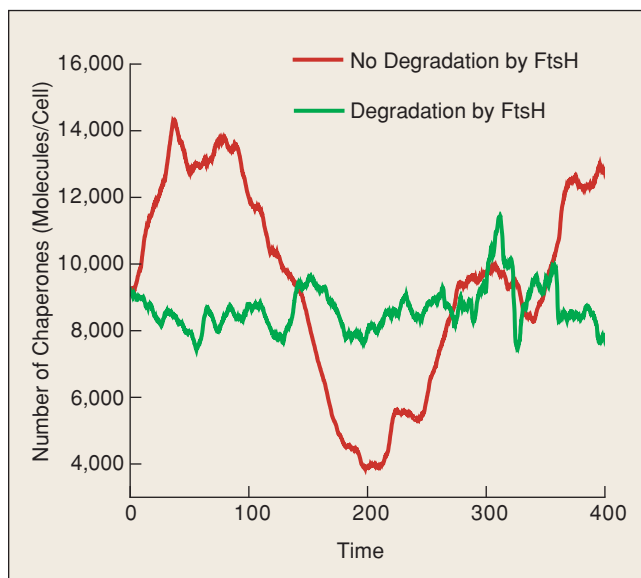


Figure 8. Stochastic level of chaperones in the presence (green) and absence (red) of the outer degradation feedback loop. The use of feedback in regulated degradation of σ^{32} attenuates the fluctuations of the chaperone level around its mean. The simulations use the Gillespie stochastic simulation algorithm [39].

lated as a necessity and identified in the physical process thereafter. The ubiquitous need for perfect adaptation, combined with signal detection, motivates a generalization of the internal model principle to include such classes of systems [41].

The necessity aspect can also be approached using new systematic mathematical tools for model invalidation using SOSTOOLS [42]. Using SOSTOOLS, we can investigate whether the dynamics generated by the various feedback loops in the HS system are necessary to explain experimental data. Such a question can be answered if, for example, we can construct so-called “barrier functions” that separate the evolution of the model lacking those feedback loops from measured data. The existence of such a barrier implies that the experimental data cannot be reproduced for any admissible parameter regimes in the deficient system. A concrete example investigates the degradation feedback loop in the HS system and proves its necessity in the sense explained above [32].

Sufficiency is yet another aspect that needs to be addressed in biological model building. In a biological setting, sufficiency refers to the ability of the known components of a design or its model to account for observed phenotypes. Sufficiency partly connects experimental data generated by direct measurements from a system to the dynamics that can or cannot be generated by the known components of this system. To illustrate this idea, we again consider the HS system. It has been long known that the induction phase in HS is due to the partial melting of the secondary structure of the σ^{32} mRNA, which implements a built-in RNA thermosensor that induces a sudden and sharp increase in the cellular level of σ^{32} upon HS [43]. Conversely, during the adaptation phase, the level of σ^{32} declines until it reaches a level three to five times higher than its low temperature level. This decline was thought to be the result of a shutoff of σ^{32} synthesis caused by either the return of the mRNA to its original closed configuration or by a feedback mechanism orchestrated by the HSPs that negatively regulates translation efficiency [44]. This hypothesis was a widely accepted interpretation as the only possibility for the observed shutoff and, as such, was part of the working molecular model for HS. The arduous search for such a mechanism was never successful, however. A simple use of the dynamic model developed for the HS response would have determined that the known dynamics of feedback in the HS system are sufficient to reproduce the observed adaptation without the need for a translation shut-off factor. Such a conclusion is instantaneously achieved by simulating the model with and without the shutoff and observing that the model shows a decrease in the σ^{32} level without decreasing the translation to its initial value. These results agree with the experimental results of [45], which prove the absence of this mechanism.

The Chemical Master Equation and the Gillespie Algorithm

The chemical master equation (CME) accounts for the probabilistic nature of cellular processes. The CME describes the time evolution of the probability of having a certain number or concentration of molecules, as opposed to a deterministic rate equation that describes the change in the concentration of these molecules. In the master equation, reaction rates are transformed into probability transition rates, which can be determined based on physical considerations. The CME can be derived based on the Markov property of chemical reactions. In this formulation, we consider a chemically reacting system involving N molecular species S_1, \dots, S_N reacting through M reaction channels R_1, \dots, R_M . Let $X(t) = (X_1(t), \dots, X_n(t))$ be the state vector, where $X_i(t)$ is a random variable that defines the number of molecules of species S_j in the system at time t . We assume that the system is well stirred and in thermal equilibrium. Under these circumstances, each reaction channel R_k is characterized by a propensity function w_k and an N -dimensional state change vector $s_k = (s_{1k}, \dots, s_{Nk})$. The vector s_k represents the stoichiometric change of the molecular species by an R_k reaction. Let

$$S = [s_1 \quad s_2 \dots s_M]$$

and

$$W = [w_1 \quad w_2 \dots w_M]^T.$$

The chemical master equation written for the evolution of the probability distribution is given by

$$\frac{\partial P(X, t|X_0, t_0)}{\partial t} = \sum_{k=1}^M \left[w_k(X - s_k)P(X - s_k, t|X_0, t_0) - w_k(X)P(X, t|X_0, t_0) \right],$$

where $P(X, t|X_0, t_0)$ is the probability that at time t , $X(t) = X$ given that $X(t_0) = X_0$, where X and X_0 are integers.

In general, the chemical master equation is analytically or numerically solvable only in the simplest cases. Therefore, one has to resort to Monte Carlo simulations to produce sample paths of the system under study. We briefly describe the Gillespie algorithm as the most commonly used stochastic simulation method. Starting at time t , the algorithm samples the time τ to the next occurring reaction from the exponentially distributed random variable with mean $1/w_0(X)$, where $w_0(X)$ is given by

$$w_0(X) = \sum_{k=1}^M w_k(X). \quad (9)$$

The algorithm also determines the next reaction R_k to occur as the one whose index k is the integer random variable with probability $w_k(X)/w_0(X)$. Based on τ and R_k one can then advance the simulation time by τ , update the state of the system, and repeat until final time or state is reached. The trajectory obtained in this fashion is a stochastic realization based on the description of the master equation. The Gillespie stochastic algorithm tracks all the reactions that occur in the system and the species they affect.

Complexity and Robustness

A functional criterion, universally present in man-made and naturally occurring systems, is the need for robustness. Whether designed or evolved, these systems need to be competitively robust in uncertain environments. Technologies and biological mechanisms that suffer from unremedied fragilities to frequently occurring disturbances in their environment are bound to be surpassed [46]. Battling such fragilities in engineering systems through the use of feedback has a rich history, starting in antiquity with simple schemes of flow-rate control to regulate water clocks and extending to recent times, where machines, such as airplanes, possess computers to regulate various functions. The use of elaborate and increasingly sophisticated control mechanisms results in more

reliable systems, all the while generating spiraling levels of complexity. It is becoming increasingly apparent that robustness—implemented through complex feedback loops and structures, rather than through the use of precision components—is also a salient feature of biological organization. Consider, for example, the HS system where the presence of feedforward, sequestration, and degradation feedback loops is justified by the need for robust operation in the presence of parameter fluctuations and intrinsic biochemical noise. Note, however, that this need for robustness is balanced by constraints resulting from other performance criteria, such as the transient response and the limited cellular energies and materials. These constraints reflect tradeoffs similar to those encountered in the design of engineering systems.

Modularity and Model Reduction

In addition to robustness, structural features of biological networks present unique challenges for modelers. For example, biological networks often exhibit time-scale separation whereby chemical reactions evolve at drastically different rates, in addition to concentration scale separation, with species molecular counts ranging from a few to millions of copies per cell. In addition to hierarchical modes of control with quantities (states) decoupled from the operation of the network through robust regulation upstream, these features render biological networks amenable to reduced-order descriptions through various approximations, such as singular perturbation, applied to the detailed mechanistic description of those systems. This possibility motivates a renewed look at model reduction techniques whereby such features are exploited in a more systematic setting. For example, the HS response can be shown, through the use of the principles described above, to be collapsible into a three-state model that captures the core functionality of the full order system [36]. Models for various other biological systems have been shown to be reducible through similar principles [47], [48], indicating that such an endeavor can generate general schemes for biological model reduction.

Stochasticity and Feedback

Living systems are inherently noisy. In addition to their exposure to environmental (extrinsic) noise, these systems experience biochemical (intrinsic) noise, leading to fluctuations in their molecular species. The magnitude and nature of these fluctuations are thought to depend on the structure of the molecular networks, the concentrations of the molecules that populate this structure, and the reaction rates of the underlying biochemical reactions. At the same time, these systems are expected to function reliably and even thrive in the presence of noise. Robust operation in the presence of noise and fluctuations is in part the outcome of feedback regulatory loops. Complete experimental and mathematical evidence of this hypothesis is lacking. By adding external additive or multiplicative Gaussian noise in the deterministic rate equations, it is possible to assess the benefits of regulatory loops by computing an appropriate performance measure. Although this exercise is common practice in stochastic control, one has to keep in mind that this modeling approach does not generally account for the intrinsic stochastic fluctuations inherent in biology. Therefore, one must study the evolution of complete probability distributions rather than first and second moments, which completely characterize the Gaussian distribution. The role of feedback in noise rejection is considered in [35] for a linear model of gene expression in prokaryotes. In this case, computing the moments of the resulting steady-state distribution is possible. For most realistic cases where the propensity functions are nonlin-

ear functions of the species concentrations, however, such an exact investigation is difficult. Therefore, the problem of studying such systems and the role of control in their operation still awaits the development of new methods and approximations. It is worth mentioning that noise rejection is not the only possible behavior that can be expected from a stochastic investigation of biological systems. There is strong evidence that some biological structures amplify and exploit noise to achieve various useful functions. For example, stochastic focusing, as generated by standard hyperbolic inhibition, can make a gradual response mechanism work more like a threshold mechanism [49]. This example shows that rigorous methods can be essential for uncovering the range of exotic behavior expected from biological dynamics.

Conclusions

The discovery of DNA and its essential role in cellular function has revolutionized the science of biology. Rapid progress in biology has led to new and exciting discoveries that promise to uncover the basic underpinnings of life. As biology becomes more quantitative, the role of mathematics becomes more important. Furthermore, the complex nature of biological systems makes an integrative systems approach essential.

In the technological sciences, particularly engineering, systems approaches have been extensively used to analyze and design man-made systems. A central unifying theme is the concept of feedback. Despite the fact that feedback control mechanisms abound in biological systems and are behind much of their complexity, relatively little work has been done to understand these complex mechanisms using ideas from systems and control theory. The study and understanding of regulation mechanisms in biology presents a unique opportunity for control scientists. One example can be drawn from the important field of endocrinology. While many hormones have been identified to play a role in regulation, feedback mechanisms are rarely studied in the context of dynamical systems. Statistical methods consisting of measuring variables and correlating them to observed behavior at a given time cannot explain the entire picture because they ignore the dynamic nature fundamental to almost all feedback systems. Only by understanding the dynamics of the underlying hormonal regulation mechanism, as captured by mathematical models, can one provide complete explanations and make predictions.

The situation is similar at the cellular level, where biochemical networks possess elaborate regulatory mechanisms that allow the organism to adjust to its natural milieu and cope with the occurrence of extraordinary and novel conditions. The logic and physical implementation of these networks include sophisticated interactions, time delays, positive and negative feedback, and crosstalk. It is becoming increasingly apparent that interactions between these ele-

ments and the complex dynamics that result from these interactions cannot be solely captured through casual intuition.

One reason for the relative absence of engineering approaches in the biological sciences is the large differences in culture, approach, and tools used in these fields. However, with new discoveries at the cellular level, the availability of new methods for collecting data, fast computers, and new theories for simulating and validating models based on these data, the time is ripe for a joint research effort. This effort can in turn provide the important and necessary link between what is known at the component and system level and what is observed experimentally.

In this article, we focused on two areas where control theory can play a key role. However, there are many areas of potential interplay between biology and control theory including neuroscience, ecology, organismal biology, cell biology, and molecular biology. But no matter which aspect of biology is addressed by control scientists, it is clear that fruitful research requires collaborations with researchers in the biological sciences. Large cultural differences between the two fields must be overcome, and the research must be relevant biologically, and not superficially so. The fruits of multidisciplinary collaboration are already visible. Mathematical modeling has not only helped reverse engineer biological systems, but it has also made forward engineering possible. Examples are numerous. For instance, molecular switches and biological oscillators are less obscure objects due to various models that elucidate the interplay between negative feedback and positive feedback loops in explaining their behavior [41], [50]. Switches, oscillators, and logic gates have been designed using engineering principles and constructed using genetic fragments and cellular components [11], [51]. Much is yet to be explored and learned from the richness of biological phenomena, however. If this opportunity is seized, control theory becomes the bridge between the technological sciences and the life sciences. Indeed, systems and control scientists are well positioned to make important contributions and take part in what promises to be some of the most exciting scientific discoveries of the new century.

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