How Do Control-Based Approaches Enter into Biology?

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Abstract

Control is intrinsic to biological organisms, whose cells are in a constant state of sensing and response to numerous external and self-generated stimuli. Diverse means are used to study the complexity through control-based approaches in these cellular systems, including through chemical and genetic manipulations, input-output methodologies, feedback approaches, and feed-forward approaches. We first discuss what happens in control-based approaches when we are not actively examining or manipulating cells. We then present potential methods to determine what the cell is doing during these times and to reverse-engineer the cellular system. Finally, we discuss how we can control the cell’s extracellular and intracellular environments, both to probe the response of the cells using defined experimental engineering-based technologies and to anticipate what might be achieved by applying control-based approaches to affect cellular processes. Much work remains to apply simplified control models and develop new technologies to aid researchers in studying and utilizing cellular and molecular processes.

Keywords

cells, molecules, control theory, feedback, input-output, nonlinear, control integration
1. INTRODUCTION

Understanding the intricacies of cellular regulation has been hampered by the inability to probe and model cellular dynamics using systems-based approaches (black-box, gray-box, or clear-box approaches). These approaches have been extremely challenging owing to the absence of experimental systems and cell-scale actuators that can exert real-time external control of particular cellular processes with the required spatial and temporal resolutions. These limitations also are influenced by the lack of ability to acquire quantitative long-duration, input-output time-series data that represent the dynamic signals used by biology for sensing and control. These dynamics are critical in numerous biological areas. Just as the study of dynamics in organs has been critical to the understanding of physiology, the study of dynamics in cell processes is necessary for understanding cell function, organism development, and disease. For example, in whole-organ physiology, control has been modeled in detail to provide a clear understanding of a multitude of phenomena such as the control of blood oxygenation, pH and pressure, body temperature, heart rate, hormone and glucose levels, neurohumeral feedback, pain adaptation, skeletal muscle contraction, and visual object tracking (1–3).

Through the knowledge gained from these models and the application of it at the cellular level, numerous novel approaches have begun to lead us toward exciting advancements, especially in the area of control and dynamics. As one example in which technologies have advanced the understanding of dynamics, the development of voltage-clamping techniques led to the first complete quantitative description of the factors and processes that control the resting and action potentials of electrically active cells (4–6). Despite early successes, though, quantitative control has unfortunately played a minor role at the level of individual cells. Although the concepts of feedback and feed-forward are commonly utilized in the discussion of genetic and signaling networks, there has been a reliance on qualitative reconstruction of detailed biochemical pathways with only limited support by modern control theory. The great news is that this situation is changing with the appearance of quantitative models of complex biological processes—for example, the cell cycle (7) and glucose metabolism in yeast (8), among others. We strongly believe that this trend will continue as the merits of control-based approaches such as control theory (9) and frequency domain analysis (10, 11) become better appreciated and the role of common biological strategies and functional modules becomes better defined (12–15). As we describe here, though, applications of
control theory go beyond understanding how cells control themselves. Control theory is equally applicable in allowing us to take external control of particular cellular processes that ultimately may result in great advances that would not be possible when using other approaches. The results of these types of approaches may help in areas from cellular production of biopharmaceuticals to biofilm control to novel cancer treatment approaches. Control can, for example, allow us to optimize, either in an experiment or in production, energy consumption, behavior, cost of reagents, speed of production, cost/benefit ratio, response time, time between failures, volume, cost per unit product, entropy, or system stability.

The ability to control and monitor the inputs and outputs of these biological black-, gray-, and clear-box systems is an extremely challenging goal. Some work has been accomplished in this area, but with limited applications. This work parallels advances that initially were made in the control of black-box nonbiological systems. For example, a multiple-input/multiple-output (MIMO) control approach, which we describe in terms of biological application in this review, was used by the aerospace and other industries to revolutionize design, manufacture, testing, and production. Unfortunately, these approaches are quite different from what could be immediately used in biology, where feedback and control are both intrinsic and extrinsic and represent biological control of and response to both intracellular and extracellular conditions. Hence, many existing methods of dynamic systems and controls may have limited applicability to biological systems; this calls for the development of an expanded theoretical framework that we term biological multiple-input/multiple-output (BioMIMO) control (Figure 1).

Control theory already has been used to successfully model small, “soft,” room-temperature systems in addition to electrical, mechanical, and chemical process technologies. These uses of control theory as an interdisciplinary science go back at least several decades. For example, in 1959, Belousov (16) first reported temporal oscillations in a reaction involving [Ce(III)/Ce(IV)], and subsequently the development of spatial as well as temporal structure in this reaction was reported by Zaikin & Zhabotinsky (17). Building on this work, Field & Noyes (18) provided a detailed mathematical analysis of how these temporal and spatial oscillations may arise. These early yet fundamental and extensive reviews of the field of oscillatory chemical reactions are useful in the area of control theory applied to nonobvious systems (19, 20).

Cells are in a constant state of feedback and response to numerous stimuli, some of which are self-generated. This often is in contrast to the status of an industrial plant or aircraft, which may change with time, whereas the cell is actively controlling and modifying its environment at both the intracellular and extracellular areas generally for its own benefit. Control challenges for cells also extend over multiple scales in space and time, with some cells being affected at long distances by other cells through paracrine signals such as hormones. The span of time also is a significant factor, as genetic regulation requires times that are orders of magnitude longer (e.g., hours = \( \sim 10^4 \) s or days = \( \sim 10^5 \) s) than the times of biochemical responses, such as calcium with direct contact of a receptor-ligand pair, which can occur in the millisecond time frame. This process of feedback and control is universal, and as it affects single-cell microbes to complex multicellular systems, it could be considered a condition in the definition of life.

In this interdisciplinary area, control engineers and cell biologists are increasingly collaborating as they recognize similar attributes in the systems they study, such as amplification, positive and negative feedback, regulation and control, oscillatory and nonlinear behavior, oscillatory and bistable behavior, disturbance rejection, noise rejection, redundancy, and robustness. In many instances, the vocabularies of the engineering and biology domains are the same with regard to dynamic systems. However, one significant difference between the two domains is that there is generally no distinct control unit for a particular biological control or regulation process (i.e., the control unit consists of fully embedded molecular reactions, cellular microdomains, single cells,
or multicellular interactions). In biology, feedback control is implicit and spatially distributed within the cell or tissue, as proteins, metabolites, and other signaling molecules are released, modified, or removed downstream in a biochemical pathway. This response, in turn, can act on processes either in a feed-forward nature on downstream components in the pathway or as feedback to upstream components. In addition, the nature of feedback and feed-forward control at the cellular level is such that one may have difficulty separating cause from effect. For example, is it the initial stimulus that causes upstream signaling to feed-forward signaling, resulting in altered downstream signaling? Or is it feedback from downstream signaling that feeds back to upstream signaling events to then alter their subsequent signal transduction (which eventually changes the
downstream signaling)? Therefore, as we study biological control, we must be mindful that we are studying emergent phenomena that are both time regulated and space regulated and comprise a living system. We also point out that we are not presenting an exhaustive review of control within and of biological systems, but instead a motivation for further application of control theory to biology. We also apologize to the authors of the many fine papers we are unable to cite because of space constraints.

2. INPUT-OUTPUT APPROACHES IN BIOLOGY

Although control processes may be implicit, the concept of inputs and outputs is still valid at the cellular level, particularly with regard to extracellular stimuli and externally manifested responses or distinct regulatory modules (Figure 1). Cells continuously receive inputs from a variety of stimuli while simultaneously producing various outputs in terms of their internal state as well as their surrounding environment. The inputs can include electrical activation, scaffolding structure, heat, the local chemical or mechanical environment, and signaling molecules (21–26). Outputs may include the release or modification of signaling molecules; internal metabolic or catabolic changes; changes in cell structure, morphology, or sensitivity; movement; stimulation or retardation of the cell reproductive cycle; or even apoptosis (programmed cell death). Additional complicating factors in the control of biological systems are the nonlinear, stochastic, and redundant properties of cellular signaling pathways. For example, many cellular responses arise from high-order nonlinearities that create sharp thresholds, but not to the extent that Boolean logic explanations alone suffice. Similarly, gene transcription within a cell can be regulated by fewer than ten copies of a regulatory molecule, so that control can be truly stochastic. Furthermore, knockout of a particular regulatory pathway can be compromised by the upregulation of a parallel and redundant alternative pathway. Questions about optimal control and estimation that need to be asked include whether the biological system and the experimental system manipulating the cell are controllable, observable, stabilizable, and detectable (27). Hence, the application of modern feedback and control theory offers a remarkable opportunity for biologists and control engineers to work together to advance our knowledge of and capabilities in biological sensing and regulation. This review describes the foundation required for constructing such an understanding (Figure 1), which in turn will enable enhanced control of biological processes and systems for medical, scientific, ecological, and industrial applications.

Figure 1
Control-based approaches from engineering to biology. Multiple-input/multiple-output (MIMO) control for cellular and engineered systems. (a) The diversity of input stimulation and environments that can be controlled as inputs to living cells. Cells will respond with an even greater diversity of detectable outputs. Cells inherently have feedback and control, which are part of the link from the input stimulation to the output responses, and experimental biology benefits from matched sensors and actuators. Many similarities exist between this approach in biology and well-established practices in fields such as robotics. (b) Robotic MIMO control/feedback loops. Autonomous control of vehicular robotics (e.g., “Boss” from Carnegie Mellon University) has many parallels to internal control of cells. (c) Cellular biological multiple-input/multiple-output (BioMIMO) control/feedback loops. A simplified gene regulatory network for galactose utilization in yeast suitability for external control of yeast metabolism. The extracellular input is \([\text{glu}]\). The natural outputs of the network are concentrations of various proteins and protein complexes (g), determined by the corresponding mRNA levels (m) that can be detected through the inclusion of a different green fluorescent protein in each gene. Adapted from Yang et al. (158), as redrawn from Bennett et al. (161).
Whereas input-output processes often are linked directly to a variety of disease states that often result in their identification, understanding the response of the cell requires both experimental and theoretical approaches focused on fundamental cell biology. However, the probing of the dynamics of cellular processes using black-box approaches, which do not consider the detailed intracellular mechanisms, has been extremely challenging owing to the absence of experimental systems to acquire long-duration, quantitative, input-output time-series data (28). The dearth of technologies for quantitative high-throughput experiments and for the application of MIMO control theory to cells is attributable to the small size and complexity of cells, the inability to measure the concentration or activity of more than a few protein species at a time, and the paucity of intracellular actuators that can be controlled externally. For example, an adherent mammalian cell may be only approximately 30 \( \mu \text{m} \) in diameter and 5 \( \mu \text{m} \) high but can contain on the order of \( 10^9 \) molecules.

The ability to actuate cellular systems (inputs) is important in control-based approaches (Figure 1). We briefly introduce a selection of the inputs and outputs here and provide additional detail on these in Section 3. One of the earliest intracellular actuators (input approaches) was the voltage-clamp micropipette, which led to a revolution in cellular electrophysiology. There have been numerous other clever input approaches developed in recent years, including caged molecules, which provide release but not the removal of molecules such as oxygen, adenosine triphosphate (ATP), and neurotransmitters such as gamma-aminobutyric acid (GABA). Although they are a great example of an actuation, they unfortunately cannot serve the further purpose of acting as reversible or bidirectional actuators (29–33). Reversible isomerization of synthetic photochemicals ultimately may provide reversible control of other biomolecules (34–38). Optogenetics, which offers the possibility of reversible control of ion channels, pumps, and enzymes, also may lead to a revolutionary advance in the ability to control cells and understand how cells control themselves. The situation for application of extracellular control and sensing is improving with microfluidics, electrochemical sensors, and ion mobility–mass spectrometry (IM-MS), for example, offering real-time control and sensing of the extracellular environment (39).

In addition to understanding the inputs, understanding how cells are responding from an output perspective is critical. There have been significant advances in the output area, including the ability to monitor dynamic cellular response. The use of the intracellular expression of green fluorescent proteins (GFPs) and their derivatives has revolutionized our understanding of protein production and modification, for example. This approach, though, is limited, as only a few colors can be examined at one time and the conjugation of these reporters to proteins can affect the properties of the protein under study. Other approaches are extremely useful as well, including intracellular fluorescent dyes and even some genetically engineered proteins, which can report on transmembrane potential, intracellular calcium, secretion, and metabolic and signaling processes. Again, though, these output signals can be probed only for a limited number of colors at one time.

All these approaches are important, and integrating them into a controlled input-output-based BioMIMO system will be extremely challenging. In this vein, a lack of adequate integrated, quantitative experimental and corresponding theoretical systems is already hampering systems biology and other biological/medical sciences. BioMIMO will require black-box effective models or coarse-grained modeling approaches, as well as the introduction of larger numbers of simultaneous sensors and actuators than are currently in use. Furthermore, the goal of applying dynamic systems controls should be not only to understand cellular regulatory and control mechanisms but also to seize external control of multiple cellular functions so that cells might operate at points beyond their normal envelope. This has to be done with the recognition that cells control their environment and also that the environment controls the cells. The duality of feedback and response between cells and their environment is a critical point when thinking not only about how
to understand cells but also about how to use their inherent abilities to produce new approaches and applications. For example, the ability of a small number of stem cells to secrete factors in response to local signals and thereby control the differentiation and remodeling of a larger number of cells in the failing heart or wounded skin would provide a tremendous advance in many areas, as the stem cells are controlled by the environment but also, over time, exert control over the environment (40). We need to develop the ability to simultaneously and actively control both the cells and their environment in terms of inputs and outputs; this will represent a major challenge for experts in both control and biology.

3. HOW DO WE FIGURE THIS OUT? WHAT IS NEEDED TO REVERSE-ENGINEER THE SYSTEM?

3.1. System Identification Approaches

In this section, we present common control-based approaches from an engineering perspective to provide insight into how to apply these engineering principles to biology. Engineers often use system identification when examining the dynamics of a system—an approach that already has been extremely useful in areas such as robotics (e.g., 41).

Engineers typically approach the problem of identification of a system’s dynamics in one of three ways:

1. A black-box approach where no internal details are assumed about the functioning of a system, and a mathematical model is derived from input-output behavior alone. (See, e.g., References 42 and 43.) The resulting mathematical model for this system, however, may have no relation at all to physical quantities internal to the system. An example of this approach is the modeling of the input-output characteristics of the head positioning of a disk drive from frequency response measurements. The system exhibits multiple lightly damped oscillations due to complex vibration modes in the mechanical system. The model provides only the parameters of the input-output differential equation, with no indication of which vibration mode corresponds to which oscillation (44).

2. A gray-box approach where there is a model of the system with unknown parameters, and input-output behavior is used to determine those unknown parameters. (See, e.g., References 45 and 46.) The model includes salient features of the physics of the system but makes assumptions such as some components changing slowly and therefore approximated as constant, some components equilibrating rapidly and therefore approximated as algebraically related to other quantities of interest, or some components having small effects and therefore essentially being decoupled from the dynamics (45, 46). An example of this approach is in the identification of parameters of the dynamic model of a robotic helicopter from MIMO frequency responses where the form of the model was derived from first principles, but its parameters were too difficult to determine from characteristics of the individual components of the aircraft (47).

3. A heavily reductionist or clear-box approach where all internal details and dynamics are modeled. An example of this approach is the modeling of nonlinear dynamics of a robotic manipulator using Lagrangian dynamics (e.g., 48). Here, the masses and moments of inertia of the individual links are combined in a sophisticated set of coupled, nonlinear differential equations to predict robot movement under the influence of motor inputs, gravity, and disturbances. For biology, this may be an extremely challenging approach because the interactions of millions of molecules (a million raised to the millionth power) must be known. A totally realistic biological simulation of a mammal might involve Avogadro’s number
of partial differential equations, termed a Leibniz (49) (such a calculation obviously is not achievable with today’s digital computers but is performed by an animal’s analog computer in real time). Even if all these connections are known someday, the prediction of cell response will remain formidable owing to the fact that the highly structured interior of the cell is crowded and heterogeneous, resulting in localized spatiotemporal dynamics. Fortunately, one does not need to know all the details to understand and learn to modify important parts to alter system function in desirable ways.

In terms of which methodology to use in biology, the clear-box approach would be most suitable for simulation studies of a system where the characteristics of the components and their interactions with other singular components are well understood but the collective behavior is unknown. Although the vast increases in computing power over the past four decades have made this approach extremely useful for designing complex engineered systems from micromechanical accelerometers to aircraft, it is difficult to apply in biology. This is partially due to the complexity of the systems under study, which is so great and has so much uncertainty in the characteristics of the components that a realistic model could not be undertaken (e.g., hundreds of millions of protein molecules interacting together, with the fact that each of these proteins may have hundreds or even thousands of different isoforms).

Both the black-box and gray-box approaches have utility when not every connection is known. The black-box approach is most suitable when only input relationships are important, and the internal structure, even if roughly known, is not needed for operation of the system or for design of the control of the system. The gray-box approach is used most frequently when the system can be approximated as a linear system. In this case, system identification reduces to identifying the coefficients of the linear, constant-coefficient, input-output differential equation in the time domain, or equivalently the transfer function in the frequency domain.

The gray-box approach is suitable for systems where there is partial but incomplete knowledge of the internal functions of the system. This approach is particularly suited to determining if a model structure is correct and if this structure is likely to be of the most use in applying control theory to biology. Here, biology can provide information or hypotheses about the topology of the dynamics—that is, which components interact with one another and whether those interactions activate or inhibit processes. The system identification can determine the rate constants or even the validity of the topology.

3.2. The Three Fundamental Types of Feedback Control

Cells are universally known to exhibit feedback control (e.g., 9, 12, 50), which allows the cell to sense inputs and respond through altering their internal functioning, including signal transduction pathways. There are, in general, three fundamental types of feedback control, which have been applied to a diversity of nonbiological challenges and are useful for biological studies: proportional control, derivative control, and integral control. Understanding the properties of these three fundamental types of feedback combined with input-output behavior from experiments can lead to important insights into how biological control systems work.

3.2.1. Proportional control. In proportional control, the control action at a particular moment is proportional (perhaps nonlinearly) to the “error” at that moment, where the error is the difference between a reference quantity or signal and the actual output of the system,

\[
\text{control action} = f(\text{reference quantity} - \text{actual output}),
\]
where \( f \) indicates a function quantity in the parentheses. In engineering, the function is usually just multiplication by a constant:

\[
\text{control action} = \text{constant} \times (\text{reference quantity} - \text{actual output}).
\]

A simple, everyday engineering example is the cruise control in a car. The reference quantity is the desired speed of the car and the actual output is the actual speed of the car. The speed error is positive if the desired speed is greater than the actual speed, and the error is negative if the desired speed is less than the actual speed. A cruise control using proportional control would apply a throttle that is proportional to this error.

An important limitation of proportional control is the phenomenon of steady-state error; that is, in a system requiring nonzero control input to maintain the system’s output at the reference quantity, proportional control alone cannot maintain the actual output at the reference quantity for any extended period of time. The cruise control example is illustrative. Suppose the desired speed of the car is 100 kph. If the actual speed of the car is 100 kph, then the error would be zero. Because the throttle is proportional to the error, the throttle input would be zero, and the car would begin to slow down. The desired speed cannot be maintained with proportional control alone.

### 3.2.2. Derivative control

In derivative control, the control action depends on the instantaneous rate of change of the error:

\[
\text{control action} = f(\text{time rate of change (reference quantity} - \text{actual output)}).
\]

Derivative control can speed the response and enhance the stability of control systems. In engineered systems, it is always employed with proportional control (PD control) or with proportional control and integral control (PID control). Derivative control requires the “computation” of the derivative of the output, which in engineering systems is done with analog circuits or on microprocessors. How a biochemical reaction might compute a derivative is unclear, although Ličko (51) suggests one possibility by release of chemicals from vesicles.

### 3.2.3. Integral control

In integral control, the control action depends on the time history of the error. The control action is proportional to the integral (or accumulated) error. Integral control can eliminate steady-state error:

\[
\text{control action} = f \left( \int (\text{reference quantity} - \text{actual output}) \right).
\]

Returning to the cruise control example, a cruise control employing integral control would apply a throttle that is proportional to the integral of the error in speed over time. As the car’s speed approached 100 kph, the error would decrease to zero, but the integral itself would remain positive. Because the throttle is proportional to the integral or accumulated error, the speed would be maintained at the desired value. It is clear that many biological mechanisms must employ integral control for homeostasis (e.g., endotherms and body temperature), even if the biochemical mechanisms implementing this control are unknown. For example, a nonzero metabolic input is needed to maintain a set-point temperature. If proportional control alone were employed for temperature control, then there would be persistent deviations from a constant body temperature as the temperature of the environment changed, and the size of these deviations would depend on the temperature of the environment.

A limitation of integral control is the speed of reaction compared with proportional control. The moment the error appears, proportional control provides a control action, whereas the integral
will still be zero. The error must be present for some time for the integral control to provide a control action. Another limitation is that integral control can lead to undesirable oscillations and even instability (52). For these reasons, proportional control and integral control are often used in tandem in engineered systems, the combination of which is known as PI control. Proportional control provides a fast response to an error, and integral control ensures that there will be no steady-state error.

Although not a purely cellular example, a particularly elegant example of using the properties of proportional and integral control for system identification in the calcium homeostasis mechanism in dairy cows at parturition is provided by El-Samad and Khammash (53, 54). The basics of their applied approach provide a platform to use in more direct cellular control-based approaches. By observing that plasma calcium levels rapidly return to set-point values after the rapid increase in demand for calcium for lactation (i.e., achieve zero steady-state error), they deduce the presence of integral control in the hormonal control system. Using known endocrinology of calcium fluxes from bone and from the intestine, they show that the calcium homeostasis system is a proportional integral controller.

Biological systems often exhibit variations of proportional and integral control where the parameters of the controllers themselves change over time (55). Such variations are termed adaptive control. Model predictive control is a method of control that has been applied successfully in process control (e.g., petroleum refining), where a model of the process is mathematically inverted to determine the control signal (56). This approach has been employed in therapeutic applications and for external control of biological systems. However, it does not appear that biological systems do (or even could) exhibit this kind of control behavior.

4. HOW DO WE SEIZE CONTROL OF THE CELL?

4.1. Cellular Input (Actuator) and Output (Sensor) Techniques

Now that we have discussed how control-based approaches are involved in cellular functioning, we next examine how to seize control of the cell. Especially for BioMIMO, the lack of techniques to seize control of a cell through imposing inputs (actuators) and then measuring biological output responses (sensors) with sufficient temporal and spatial resolutions over long enough periods of time has hampered the ability of engineers to contribute to biology. Biological systems are fundamentally nonlinear, displaying classic hard nonlinearities such as saturation, dead zones, hysteresis, and threshold triggers (threshold detection). Dynamic nonlinearities include bistability, limit cycles, and chaos. However, simulation of the coupled, nonlinear differential equation models of certain biological systems shows nearly linear response over a relatively large range of inputs (57). Biological responses can be blindingly fast, on the order of nanoseconds to microseconds for individual ion channels, tenths of a millisecond for calcium signaling, hours for gene expression, or days to years for organ formation. Manipulation of more than one input with the simultaneous measurement of more than a few outputs is presently impractical, motivating the development of novel systems for acquiring time-series data to support BioMIMO models. Numerous technologies exist for singular spatiotemporal stimulation of biological systems, including patch clamping, micropipetting, stretchable substrates, optical control of ion channels, and laser microsurgery (23, 58–62). These techniques are useful for examining local domains but provide a limited ability to stimulate and assess overall cell responses. Areas that have been evolving in this field include fabrication techniques at the organic-inorganic interface where these approaches can regulate the material-cell interactions (63–66). Whereas these systems provide numerous advances in meeting the challenging problem of interfacing man-made input stimulation systems with biology, other
techniques can provide spatiotemporal control, which will contribute to input-output analysis methodologies.

Here, we review a select number of inputs (actuators) used to control cellular environments affecting cellular response. We then discuss a selection of output approaches that are used to capture spatiotemporal responses. We describe methods that are used mostly for single-input/single-output (SISO). Although we recognize that there is a diversity of input and output approaches, we focus on a select number: For inputs, we discuss chemical, mechanical, electrical, genetic, and optical inputs; for outputs, we discuss optical microscopy, including GFP, fluorescence resonance energy transfer (FRET), calcium signaling, and genetic engineering. A discussion of techniques that could be used for MIMO control follows. Although MIMO systems are critical for the future of this approach, they are currently lacking in significant numbers, but it is promising that this is where great advances can be made by engineers and scientists as they develop new integrated approaches.

4.1.1. Chemical input. Many of the cell’s inherent functions are strongly affected by external gradients of stimuli, which can induce localized responses. These localized chemical gradients can exist physiologically in many and varied locations. In the body, these gradients include those that guide development and wound healing, those at the interface of blood vessels as chemicals pass from the vasculature to the tissue parenchyma, and those in cellular secretions that drive the recruitment of immune cells to a site of infection or alter the fate of cells in cancer (67). The gradients need not be produced externally to a cell—tumor cells in tissue secrete into the extracellular matrix (ECM) CCR7 ligands. They also slow interstitial flow, creating autologous gradients in these ligands whereby CCR7 receptors on the same cells sense to guide themselves toward the lymphatic vessels that affect the tumor (68).

Controlling chemical stimulation for these and other applications for single cells has been accomplished through a variety of techniques. For example, many experiments on fast-moving cells such as Dictyostelium discoideum can be accomplished through direct micropipetting to create point sources of chemoattractant whose location in the petri dish can be controlled easily (69) or through microfabrication approaches to apply controlled diffusion gradients (70). Motion of a micropipette can quickly switch the chemicals to which a cell can be exposed (71, 72). Classically, passive gradient chambers have been used widely to control chemical stimulation and measure outputs such as calcium signaling and chemotaxis (73–75); these systems, however, create only static gradients. One field that shows great promise for dynamic control of both spatial and temporal chemical stimulation of living cells is microfluidics. Numerous established microfluidics techniques are ideally suited to creating and maintaining space- and time-controlled stimulation that initiates spatiotemporal behavior (76–79). Microfluidic devices have been applied in a variety of areas to stimulate single cells, including yeast, Jurkat T cells, and cardiomyocytes (80–85) and have been implemented to mediate cell population attachment as well as deliver chemical reagents to specific cell populations (23, 86–89). This is important as many cellular characteristics and processes—including cell structure, motility, and apoptosis—have been discovered to be spatially and temporally responsive to chemistry.

4.1.2. Mechanical input. Mechanical stimulation also significantly affects cellular and multicellular behavior. Although mechanical stimulation is a major input, it does not dominate over biochemical interactions, but at the same time it can create profound changes in overall cell behavior even with no change in the chemical environment (66, 90, 91). Mechanical stimulation of mammalian cells has been shown to affect many cell functions, including cell motility, apoptosis, proliferation, and protein expression (92–97). Furthermore, both the differentiation of stem cells
and the invasive behavior of cancer cells are affected by the stiffness of the surrounding matrix (66, 98).

The ability to control the mechanical stimulation input has been approached through a variety of continually evolving techniques. Devices have been utilized previously to study mechanically induced effects, including those that examine shear stress, compression, tension, and ECM stiffness (99–103). The mechanical stimulation also can be delivered passively through control of the substrate stiffness (66, 104) or substrate topology (105), or dynamically by means of global substrate deformation (97). In addition, mechanical force can be imposed locally by approaches such as magnetic beads (92), laser tweezers (106), or atomic force microscope (107) (Figure 2). For example, mechanical stimulation can be imposed through Arg-Gly-Asp (RGD)-coated ferromagnetic beads to apply forces to integrin receptors (92). Researchers also have developed parallel-plate flow chambers to exert shear stress through fluid flow on living cells (79, 100, 108). In addition, a uni-directional actuation device can impose tension or compression on cells through the stretching of an elastomeric membrane (109). Such methods have revealed information regarding cell responses, including how cells exposed to stress undergo cell shape change, alignment, and microfilament network remodeling (99, 100).

Furthermore, the input in terms of mechanical stimulation often is not even as straightforward as a cell experiencing just one type of force. Although cells may experience only one type of mechanical stimulus in controlled in vitro situations (e.g., shear or stretching), cells are known to frequently experience multiple modes of mechanical stimulation in vivo (i.e., the “MI” in MIMO), such as simultaneous stretching and shear flow. From a physiological perspective, the stresses that a cell experiences depend on its environment and location. For example, cells in the vascular system experience blood flow–induced frictional drag forces from shear stresses acting on the vessel wall as well as orthogonal steady and cyclic stretching during repetitive stimulation (110). Even with just single-mode mechanical stimulation, the influence of mechanical stimuli on the structure and function of a variety of cell types has contributed significantly to our understanding of the associated biochemical response (mechanotransduction) (100, 111, 112); protein and gene expression in cells (100, 101); and cytoskeleton, ECM, and focal adhesion complex responses (92, 112, 113). Although these are only a small sampling of controlling mechanical environments and responses, the ability to control mechanical stimulation spatiotemporally is extremely important in feedback control–based approaches (Figure 1).

4.1.3. Electrical input. Electrical input applies to a variety of cellular areas, but it is especially important in two major physiological fields: the cardiac and nervous systems. For example, electrical stimulation can affect single cardiomyocytes (83) as well as play a crucial role in determining both the damage to and the potential regeneration of neurons. In addition, electrical stimulation applied to the proximal stump of a severed nerve at the beginning of surgical repair has been shown to increase the reinnervation speed of target muscles (114, 115). Additional evidence has indicated that electrical stimulation can induce a significant improvement in the regeneration of axons in cut peripheral nerves (116).

Diverse techniques can be used to examine and control electrical inputs. One of the most common techniques in studying neural response is to probe the electrical signals using glass micropipette electrodes. Whole-cell voltage clamping with such electrodes and more recently patch clamping have been applied to a variety of cells, including neural cells, muscle fibers, cardiomyocytes, and even bacteria. Other techniques are being developed as well, often through merging the development of microtechnology with neurobiology, including the use of microfabricated structures that greatly increase the speed and efficiency with which cells can be patch clamped (117).
Figure 2
Single-input/single-output (SISO) control of mechanical input linked to signal transduction output. A laser-tweezers approach to study transcell signaling in response to localized force. (a) (Left) Phase contrast imaging with a human umbilical vein endothelial cell with a polystyrene microsphere for integrin stimulation. (Right) An image of the same cell, which has been transfected by a cyan fluorescent protein/yellow fluorescent protein fluorescence resonance energy transfer (FRET)-based Src kinase biosensor. (b) Schematic of the fibronectin-coated microsphere attached to a cell. (c) Laser-tweezers traction on the fibronectin-coated bead in the upper-right corner of the cell caused FRET responses for determining signal transduction of Src kinase (106).

In addition, Campenot chambers (118) use microelectrodes and multiple chambers to study selective exposure of cells and electrical stimulation to various biochemical treatments (119).

4.1.4. Genetic input. Genetic engineering allows the creation of cells and even intact animals in which the conditional expression of a particular gene is contingent on the presence of one or more control signals (120). For example, antibiotics such as tetracycline (121) and doxycycline (122) are
able to turn on or off genes on the basis of the presence or absence of these antibiotics. Whereas many of these actuators (inputs) are binary, providing either low or high levels of expression, precise control of transcription factors can lead to not two but three different states for embryonic stem cells (123); i.e., it can be a mistake to assume that biology is Boolean. These tools, critical in molecular biology, provide control to engineers through yet another powerful actuator, but for gene expression processes this control requires hours to days from the initial time of application. It is also possible to utilize temperature, chemistry, light, and mechanics as the controlled variable in gene expression studies (124, 125).

4.1.5. Optical input. Another mode of input to cells is through optical excitation. For example, there has been a long quest for optically controlled ion channels to enable control of electrically active cells (61, 62, 126), which has grown into a field termed optogenetics. This is a promising control technique currently applied to neural studies, in which genetic engineering is used to allow optical control of transmembrane channels, G protein receptor–controlled enzymes, ion pumps, and readouts of intracellular calcium (127). Neurons can be excited optically in millisecond timescales (128), and GFPs also can be included to cause genetic modifications and provide information as to where in vivo virus-directed modifications are being expressed. Virtually all the work to date has involved electrically active neural cells, for which bidirectional control is provided by means of optically controlled ion channels to depolarize and pumps to hyperpolarize. Miesenböck (129) presents an excellent review of the field where he points out that one can quickly imagine matching rhodopsin-related genes, derived from microorganisms, to other cells, such as cells in the immune system. Another approach is a light-addressable, actively switchable system that controls the light input through Arabidopsis thaliana (130). The ability to generalize this system arises from the protein-protein interactions, which are based on one of the most common functions in cells. This finding suggests that there could be a ubiquitous and more robust platform approach that is able to affect cell behavior through membrane-based proteins with micrometer and single-second resolution. This approach enables a response to be induced through the use of light as an input activator rather than just as a cellular readout.

4.1.6. Measuring output. Measuring the output responses must be accomplished while simultaneously controlling the input stimulations for control-based approaches to be effective (Figure 1). This is especially important as the input signals elicit defined output signals. To link these responses together using gray- or black-box approaches requires controlled definitions of the initial and boundary conditions for both the inputs and the outputs. We present in more detail a select set of important techniques that can be used to probe the cellular output responses, including GFP, FRET, calcium probes, and genetic engineering. Although we are highlighting only these approaches, this does not mean that other techniques—such as the common real-time polymerase chain reaction and Western Blotting, or the much newer online IM-MS (39)—are not appropriate or useful. We focus on these techniques because most of them are related either to the optical microscope, which is prevalent in biology labs and can enable the recording of a variety of output responses with both space and time information, or to genetic techniques also common in biology labs. These output responses are essential in pursuing methodologies with control-based approaches.

The continuing generation of many new fluorescent molecular probes has created the ability for real-time monitoring of cell signal outputs. These outputs eventually can be coupled with the input stimulation techniques described above. One important technique is GFP, where naturally fluorescent proteins originally from the fluorescent jellyfish Aequorea victoria and GFP-type proteins are transcribed to enable the monitoring of localized molecules with specificity to
subcellular regions. Another example is in monitoring the movement of GFP–endothelial nitric oxide synthase fusions in blood vessels. This approach enabled the study of regulation of endothelial nitric oxide synthase (131–133). These GFP techniques also can be augmented to further increase their capabilities, such as through FRET. The change of the separation distance and/or relative orientation between two fluorophores in close proximity results in a change in the intensity due to the FRET (134, 135). FRET has been used to monitor live cellular events with high spatial and temporal resolutions (135–137). Optical switches also can be used to modulate FRET for lock-in detection and reduction of background fluorescence (138, 139). Another powerful live-cell imaging technique for detecting output responses in cells involves monitoring calcium signaling through calcium dyes such as Fura-2 and Fluo-4 (140, 141). Calcium functions as a ubiquitous messenger in cells through regulating processes such as fertilization, muscle contraction, embryogenesis, and synaptic vesicle motion (142, 143), so it is a useful real-time output signal to monitor. In addition, there are numerous genetic engineering approaches that are continuing to evolve. Miyawaki (144) provides an excellent review of how genetic engineering can provide sensors to particular cell populations for examining depolarization, calcium, calmodulin kinase activity, F-actin/G-actin equilibrium, chloride, neurotransmitter release, and formation of precursors to endocytosis. The list grows regularly and presents continued opportunities for spatially directed sensing, albeit with limitations as to the number of different probes that can be utilized at once. It is also important to note that the genetically engineered reporters can be targeted to specific cell types, whereas externally delivered fluorescent dyes may be less specific and may target all cells in a mixed-cell population. The ability to understand cell output response will continue to be enhanced through developing new output-based approaches and then linking these responses to the stimulation inputs through systems-based approaches. These output probes will continue to be expanded as these probes become more and more prevalent. (The “father” of a number of these probes, Roger Tsien, won the 2008 Nobel Prize in Chemistry for this work.)

4.2. A Challenge for Fast Control of Multiple Dynamic Variables and Multiple-Input/Multiple-Output

Two major challenges in SISO approaches are the ability to have fast control of parameters and the ability to expand these into MIMO approaches. At the same time, these challenging issues in turn present tremendous opportunities for technology development integrated with biology. The speed of the stimulation and the imaging will limit the ability to examine cellular and molecular responses, which have high-frequency content. Chemical frequency response has been shown to directly affect gene expression, and mechanical frequency response has been shown to affect cell morphology and behavior. Furthermore, although approaches are evolving to have relatively fast control over major stimulation modes (e.g., chemical, mechanical, and electrical potential), these techniques often are limited to just a SISO type of approach. There have been some recent developments pointing toward the ability to integrate these single stimulation modes into multiple modes, although mostly these are multiple-input/single-output (MISO). For example, an approach that mechanically stimulates neurons while altering their chemical environment and measuring their voltage potential changes has been developed (Figure 3) (145, 146). Furthermore, although it does not feature dual stimulation, the framework of this approach through patch clamping would potentially enable input stimulation of both mechanical and electrical parameters while controlling chemical stimulation for three modes of controlled inputs in the future. In addition, the ability to create a system to independently and also simultaneously stimulate living cells with dual modes of mechanical stimulation (shear and stretching), which are more physiologically relevant and control the chemical stimulation (Figure 3) (147), has shown that cells can structurally integrate...
Figure 3
(a) Toward multiple-input/multiple-output control. A localized polymer indentation system to control mechanical, electrical, and chemical stimulation while monitoring the output of morphology and action potential (145–147). Abbreviations: PDMS, polydimethylsiloxane; RMP, resting membrane potential.
(b) A combined dual-input mechanical stimulation approach for both shear and strain is applied to living cells while the output of their cytoskeletal network response is monitored simultaneously. Chemical stimulation also can be controlled with this approach.
multiple input modes of mechanical stimulation that results in global nonlinear network responses (R.L. Steward & P.R. LeDuc, unpublished results). The future holds much promise as more of these types of techniques are integrated and further coupled with the ability to measure multiple outputs simultaneously. As the development of these integrated approaches expands, so will the ability to apply them to control-based approaches for biology.

4.3. How Do We Control the Cell’s Intracellular Functions Directly?

4.3.1. Open-loop approaches. Above we described our ability to apply stimulation to cells directly, but altering their internal circuitry would allow the testing of intracellular functions with respect to altered dynamics. Researchers currently employ open-loop methodology to this end, where one alters the functions of the cells, for example through drugs, toxins, gene knockouts, or small interfering RNA, and then observes what happens in an open-loop fashion; there is no closing of the system by feeding back the response to modify the stimulation for further system alterations. This open-loop approach of altering circuitry and adding stimulation has blossomed wonderfully in the field of synthetic biology. Synthetic biology engineering approaches continue to develop, an exciting example of which is biological control and feedback. Two selected areas in this field presented here are genetic and molecular assembly approaches toward synthetic biology. Genetic approaches in synthetic biology include oscillators (148), logic gates (149, 150), communication-based circuits (151), bistable switches (152), and riboswitches (153). Through many of these genetic circuit designs, a conceptual idea is generalized into a mathematical model, which is then used to examine the dynamics of the design. This approach is useful for looking for trends in the subsequent testing and revisions.

Other open-loop approaches presented here include the feedback circuit, genetic toggle switch, light-switchable proteins, next-generation synthetic gene networks, and molecular assembly systems. These approaches will continue to evolve, especially with additional focus on control and feedback theory merged in many synthetic systems. In feedback circuits, creating synthetic gene-regulating networks and combining them with theory to predict behavior would be useful in areas such as nonlinear mathematics, biochemical reaction networks, and multistable biological systems. One intriguing approach is a positive feedback circuit that feeds back and activates its own expression using mutant T7 RNA polymerase Escherichia coli (154). This feedback circuit created a bistable system where gene expression existed mainly in two modes due to the feedback, although ultimately there was a nonlinear response due to the dilution of the polymerase. Another interesting and linked approach is to create a genetic toggle switch that can be turned off and on through networks of regulatory elements (152). The switching stimulation input in E. coli that enabled the output change in the genetic patterns was designed to sense chemical or thermal changes. Other systems behaviors that have been approached in this area include multistability and oscillations, which have been shown in bacteriophages, in a Cyanobacteria circadian oscillator, and in optogenetics (described above). Many of these approaches rely on synthetic biology methodology, which, although having made significant progress, requires further improvement and integration with control and feedback theory for the next generation of advances (Figure 4a,b) (155).

Beyond genetic approaches, another direction with promise is the ability to assemble molecular components to create functional artificial molecular systems. These approaches include the creation of artificial cells through molecular assembly (156, 157). Here, the idea involves the creation of a nanofactory that can monitor the level of chemical concentration in vivo and can then respond when a deleterious amount of that biochemical is present, as depicted in Figure 4c (156). The response would trigger an enzymatic reaction to return the in vivo chemical concentration back to homeostasis. This approach would involve input signaling and output response, which
Repressor 1

Repressor 2

Chemoattractant B

Chemoattractant C

Repressor 1 0 1 1 1
Repressor 2 1 0 0 0
Repressor 3 0 0 1 0
Repressor 4 1 1 0 1
Repressor 5 0 1 1 1
Repressor 6 0 0 1 0
Sensor A 1 0 0 0
Sensor B 0 1 0 0
Sensor C 0 0 0 1

Chemotaxis toward A
Increasing $P_{\text{chemo}, A}$
Increasing Repressor 1

Chemotaxis toward B
Increasing $P_{\text{chemo}, B}$
Increasing Repressor 3

Sensing

Input

Transport

Encapsulation of enzymes

V Receptor targeting

Tissue

Output

Structural scaffold

Self-destruct

Sensing

Logic

Switches

Sensor/ receptor expression

Chemotaxis network

Chemotaxis toward C

Repressor 1

Repressor 2

Repressor 3

Repressor 4

Repressor 5

Repressor 6

Sensor A

Sensor B

Sensor C

Tunable RNA feedback

Tunable protein feedback
would feed back through a sensing mechanism, which could be applied to a diversity of areas including medical treatments, biofilms in pipeline transport, and antimicrobial peptides that inhibit pathogens. Overall, in these areas, a great push needs to be made from assembly and complex control/feedback theory toward more robustly functioning system-level responses.

### 4.3.2. Closed-loop approaches

An ultimate challenge in applying control theory to biology is not simply to understand how biology controls itself, but to understand how we can exert dynamic control on biology. Closed-loop control of cells is extremely challenging because it requires real-time readouts, the ability to have that readout cause changes in the cell, and controllers that dynamically link the two (49). Approaching this feedback from an external stimulation standpoint (i.e., closing the loop through stimulation outside the cell) is much more feasible in the immediate future (Figure 5) than will be closing the loop within internal cellular alterations (i.e., closing the loop by changing internal wiring of the cell signaling pathways). To accomplish this from an external stimulation perspective, however, one must be able to monitor the state of the cell in somewhat real time and then alter the external stimulation profile accordingly. Numerous output sensor approaches can do this, including the previously mentioned fluorescence techniques. The response of these cells then needs to be correlated to functions that send signals to the system to alter the external stimulation (such as chemical, mechanical, or electrical input stimulations). For example, a cell that is undergoing a calcium spike induced by both chemical and mechanical stimulation could then feed back the spike response information of the calcium signaling through its fluorescence indicator; the mechanical and/or chemical stimulation would then be adjusted to affect the calcium signaling (e.g., reduce the chemical agonist stimulation to stop the calcium spiking). Another approach that could be useful is using a reporter of the GAL gene in the galactose metabolic system in yeast (Figure 1). This approach could be used to monitor the cell environment and then could be activated to control the galactose input to the system (158).

Microfluidics and IM-MS offer an interesting possibility for MIMO. This approach may be particularly well suited for sensing of the cellular secretome with actuator input control of the external environment, even with delays between sensing and actuation of only a couple of minutes; this is an acceptable timescale when genetic processes are the regulator (39). Accomplishing this requires the ability of IM-MS to track thousands of proteins in near real time, in contrast to the hour or more required for liquid chromatography–mass spectrometry. This would enable a greater understanding of the system identification and dynamic response, but the challenge remains in devising a matched suite of actuators. Furthermore, in the longer-term future, closing the loop with feedback also could alter the internal function of cells, as mentioned above. Although this would be difficult, using the aforementioned genetic and synthetic biology approaches may enable the external stimulation of cells to cause specific chemical reactions to occur (through perhaps the production of a GFP, depolarization of an ion channel, activation of an ion pump, or activation of a specific enzyme). This production then could be fed back to alter a second chemical stimulation that would trigger changes in the gene expression, altering the previous response. Forms of this approach have been accomplished, but mostly using more passive feedback mechanisms that

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**Figure 4**

Using control and feedback for the next generation in synthetic biology through genetic networks and integrated molecular components. (a) Genetic approaches will push toward creating tunable genetic filters through negative feedback loops with small interfering RNA, riboregulators, and ribozymes. (b) Chemotaxis may create an autonomous mobile behavior through a synthetic genetic toggle switch. Input and logic operations then control the response (155). (c) Molecular components can be assembled for sensing the input, resulting in a controlled output response approach through potential synthetic nanofactories (156). Abbreviation: P, promoter.
A schematic representation of a hypothetical microfabricated biological multiple-input/multiple-output system. The highly instrumented microbioreactor with real-time image analysis and electrochemical sensors would provide the control algorithm with dozens of dynamic output signals reporting the metabolic, signaling, and morphological state of the cells under study. On the order of a hundred computer-controlled microfluidic valves could serve as actuators to deliver control inputs to the system to dynamically regulate the extracellular microenvironment. Optogenetics could be used to extend the control to inside the cell. A downstream ion mobility–mass spectrometer would provide near-real-time metabolomic and proteomic data from either cellular secretions or the cytosol of selectively lysed cells. A systems model would be required to drive the multiple-input/multiple-output controls. All the parts have been demonstrated individually, and only their integration remains. Abbreviations: FRET, fluorescence resonance energy transfer; GABA, gamma-aminobutyric acid; GC, gas chromatography; GFP, green fluorescent protein; IM-MS, ion mobility–mass spectrometry; MALDI, matrix-assisted laser desorption/ionization; MMP, matrix metalloproteinase; nESI, nanoelectrospray ionization; PDMS, polydimethylsiloxane; QD, quantum dots. (Figure created in collaboration with Franz Baudenbacher and David Clifel.)

are predesigned to respond to specific signals in organisms such as *E. coli*. This approach and its derivatives could enable closed-loop responses to be imposed on both external and internal components of living cells, and would allow researchers to move toward an ultimate goal of system integration where they control the behavior of cells from both internal and external perspectives.

5. CONCLUSIONS

We have discussed the ideas describing how control is an inseparable part of biology. We have discussed what cells do in their innate state (i.e., when we are not looking) and then
presented control-based approaches to help figure out how cells work and how potentially to reverse-engineer them. Finally, we discussed ways to seize control of the cell through controlling inputs and examining outputs toward closed-loop feedback control approaches. Through these approaches, researchers may envision the day when they can introduce a cell into the body that is able to sense and move to a particular location. Once at this location, perhaps through internal sensing mechanisms or external stimulation, the cell could start producing therapeutic molecules to treat diseases (e.g., cancer). This ability would require control, feedback, system integration, and a vast host of other functionalities working together cooperatively toward a common goal. Although this technology is seemingly futuristic, biology already accomplishes this goal in numerous areas, which is why approaches to harness and utilize these amazing abilities would be a tremendous advance. Prior to their implementation in human biology, though, these nature-inspired approaches can be readily applied in vitro in the laboratory to improve our understanding of biology and medicine and our ability to produce biomolecules efficiently.

Overall, of course, one must recognize that biology does not naturally achieve its remarkable capabilities with SISO through control-based approaches. On the application of control theory to biology, Miesenböck (129, p. 398) notes that complex systems are best probed, not by replicating any one particular activity pattern, but by applying whole families of perturbations which may be unapologetically artificial. The core of this argument was articulated by R. A. Fisher ([159]): ‘No aphorism is more frequently repeated . . . than that we must ask Nature few questions, or, ideally, one question, at a time. The writer is convinced that this view is wholly mistaken. Nature, he suggests, will best respond to a logical and carefully thought out questionnaire . . . .’ Statisticians recognize Fisher’s questionnaires, also known as randomized multifactorial perturbations, as the most powerful experimental design for uncovering causal relationships in networks of interacting components, and biologists are taking note.

If ever there was one, this is a call for MIMO control applied to biology, wherein more than one knob is controlled at a time. Technology has now developed far beyond what Fisher imagined in 1926, and the time is now ripe for the broad entry of control theory into experimental biology and medicine. One can easily expect the emergence of more combinatorial anticancer therapies (160), and there is a need for MIMO control theory to understand, perfect, and apply such therapies to a wide variety of areas.

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