Primer

Motifs, Control, and Stability

John Doyle*, Marie Csete

Many of the detailed mechanisms by which bacteria express genes in response to various environmental signals are well-known. The molecular players underlying these responses are part of a bacterial transcriptional regulatory network (BTN). To explore the properties and evolution of such networks and to extract general principles, biologists have looked for common themes or motifs, and their interconnections, such as reciprocal links or feedback loops. A BTN motif can be thought of as a directed graph with regulatory interactions connecting transcription factors to their operon targets (the set of related bacterial genes that are transcribed together). For example, Figure 1A shows a BTN motif that describes a part of the transcriptional response to heat (and other) stressors.

But biological networks are not just static physical constructs, and it is, in fact, their dynamical properties that determine their function. In this issue of *PLoS Biology*, Prill et al. [1] show that the relative abundance of small motifs in biological networks, including the BTN, may be explained by the stability of their dynamics across a wide range of cellular conditions. In a dynamical system, control engineers define “stability” as preservation of a specific behavior over time under some set of perturbations. The definitions of stability vary somewhat depending on the types of system, behavior, and perturbation specified [2]. For the BTN example, Prill et al. [1] study stability of gene expression levels, as modeled by a set of linear differential equations. Given interactions from a BTN motif, “structural stability” is robustness of stability to arbitrary signs and magnitudes of interactions. This is such a stringent notion of stability that it would be satisfied by few systems, yet Prill et al. [1] show that all BTN motifs are stable for all signs and magnitudes of interactions. For several other biological networks, they show a level of correlation

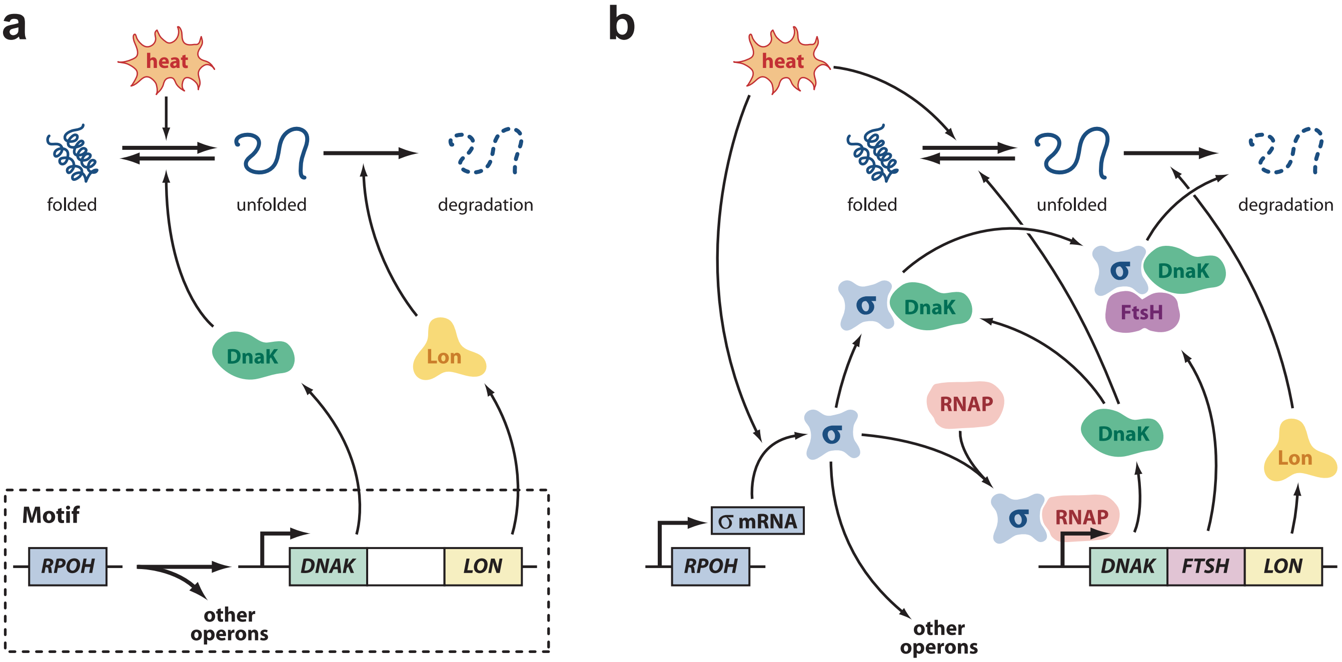

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Figure 1. Cartoons of the *Escherichia coli* HS Control System

(A) The transcriptional motif showing its basic function, the manufacture of chaperones to refold and proteases to degrade denatured proteins. For simplicity, only one operon is shown in detail.

(B) The same network including control elements. See text for explanation.

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Abbreviations: BTN, bacterial transcriptional regulatory network; HS, heat shock; PPI, protein–protein interactions, RNAP, RNA polymerase

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between abundance and structural stability that is highly unlikely to occur at random. The significance of these results as well as those in recent related papers (see references in [1], particularly those of Alon and colleagues) can be better appreciated within the larger context of well-known concepts from biology and engineering, particularly control theory [3]. For additional mathematical details underlying the qualitative arguments presented here, see the online supplement (Text S1 and S2).

Motifs, Networks, and Dynamics

Prill et al. [1] point out that their motifs are small parts of networks in at least two distinct senses, and the heat shock (HS) response will be used to illustrate these points. A motif such as that shown in Figure 1A is just one of many motifs that make up the BTN, but is also part of an even larger network involving protein–protein interactions (PPI) illustrated in the more detailed Figure 1B [4]. The HS response ultimately works on proteins—repairing or degrading misfolded proteins before they damage the cell. The small motif cartoon of Figure 1A consists of the *rpoH* gene, which encodes a transcription factor called the alternative sigma factor σ^{32}, which recognizes the HS gene promoters to induce HS-specific gene expression. For simplicity, only one operon is shown.

HS genes encode molecular "chaperones" (such as DnaK)—proteins that help refold denatured proteins—and "proteases" (such as Lon)—enzymes that degrade unfolded, dysfunctional proteins. Regulatory aspects of this motif are shown schematically in Figure 1B. Briefly, in addition to binding unfolded proteins, chaperones can also bind to σ^{32} (denoted in Figure 1 as σ), sequestering and preventing it from binding with RNA polymerase (RNAP), thus providing a negative feedback loop to modulate σ^{32} activity. The protease FtsH degrades bound σ^{32}, a negative feedback that further fine-tunes the HS response. A feedforward response is implemented in the heat sensitivity of σ^{32} translation, which is enhanced at high temperatures. These additional layers of control beyond the BTN motif alone yield a system that by engineering standards is efficient, robust, and evolvable [4]. (Note: biologists use the term "regulatory" to describe networks such as that displayed in Figure 1A, but engineers typically reserve "regulatory" for actual controlling elements as in Figure 1B.)

The motif in Figure 1A is a simple tree and is perfectly structurally stable, with its stability completely independent of the specific concentrations and kinetics of the individual molecules that compose the network [1]. In contrast, by almost any reasonable definition (including appropriately generalizing the methods of Prill et al.), Figure 1B has no structural stability, as only a small subset of possible parameter values could confer a stable network. The small motif in Figure 1A is, thus, inherently stable, but Figure 1B requires a high level of fine-tuning for stability—which, in fact, has evolved for this network. An essentially parallel story holds for other motifs, as all motifs in the BTN are structurally stable. Indeed, the entire BTN from which the motifs were extracted (without the PPI elements) is perfectly structurally stable, since it has no nontrivial feedback loops (i.e., other than self-loops, where a protein regulates its own synthesis). And to the extent that analogous PPI dynamics are known for other motifs, they too require exquisite fine-tuning for stability.

The fact that the bacterial "transcriptional networks" have such strong structural stability and that this stability is completely lost with the inclusion of protein–protein and other regulatory interactions has many possible and quite different interpretations. Structural stability is clearly not an intrinsic feature of the biology itself, but depends in a rather extreme way on the level of detail in the models chosen. Thus, based on the biology alone and the many caveats that define the way these motifs were extracted, the results based on structural stability in Prill et al. might appear to be at best speculative, and at worst misleading. We will argue, however, that their results reveal highly significant organizational principles.

Plants, Controllers, and Disturbances

Control theory uses an abstraction that is useful in interpreting biological models like those in Figure 1 ([2]; Text S1 and S2). A system like Figure 1B is often decomposed into a "plant" (as in manufacturing plant), from which the basic function of the system can be inferred, and a "controller," which implements feedback and feedforward manipulations to improve stability and robustness of this function. In this sense, robustness means that a specific plant function (such as low levels of unfolded proteins) is maintained in the face of certain disturbances (such as temperature or chemical insults). Robustness is usually used in a broader sense than stability, with the latter usually restricted to infinite time horizons and the former including additionally transient behavior and wider ranges of perturbations. Thus, "robust stability" is typically used to describe stability that is robust to some large set of perturbations. For example, in Figure 1A, heat can be viewed as the external disturbance on a plant consisting of folded and unfolded proteins plus chaperones and proteases. The controller in Figure 1B adds feedback and feedforward mechanisms to enhance robustness and efficiency in the control of unfolded protein levels, particularly in transient response to temperature change [4].

Many organizational features of this system have been experimentally studied. Removing σ^{32} (by creating bacteria lacking the *rpoH* gene) causes death of bacterial cells at high temperatures [5]. But this lethal knockout can be rescued by constitutive overexpression of the HS operons, essentially implementing the motif in Figure 1A as the complete system

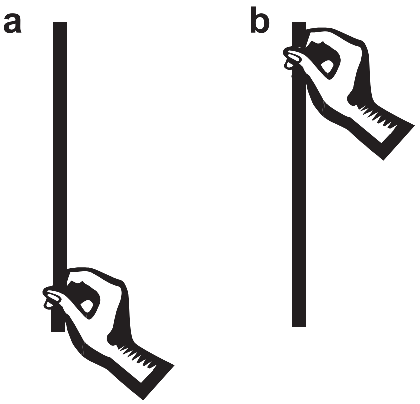

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Figure 2. Pendulum in Up and Down Positions
(A) Pendulum held in up (unstable) position
(B) Pendulum held in down (stable) position

[6]. Thus, in principle, a network with the topology in Figure 1A is viable, provided it is implemented appropriately. The controller in Figure 1B enhances robustness and efficiency but is not required for basic function, and other less complex control schemes could be used, but with degraded robustness and performance compared to wild type (nonmutants) [4]. Again, to the extent that details are known, this is a common story for BTN motifs in general [7], in which a transcriptional motif provides a core plant that performs some basic function. The actual biological network, however, often has a controller involving PPI or other mechanisms, typically of much greater complexity than the motif itself, and which provides additional robustness, efficiency, and flexibility. In engineering design, controller or plant decompositions can be nested, with one plant plus controller collectively functioning as the controller for another plant, and so on. Far more complex layered control strategies are commonly used in designing technological systems, and are presumably ubiquitous in biology. For example, the hierarchical organization of bacterial regulation includes such elements as stimulons, modulons, regulons, operons, PPI control elements, etc [7].

Such decompositions are not unique, and plant and controller can, in principle, be arbitrarily chosen components, but the choices are typically used to highlight particular organizational features of the complete system. For example, Figure 1B can, instead, be decomposed into a plant consisting of just the folded or unfolded protein levels of the cell, with heat disturbance, and a controller consisting of the entire bottom part of Figure 1B. This decomposition is more natural from an engineering perspective, but highlights the BTN motif less than when Figure 1A is viewed as the plant plus disturbance. Note that because the plant motifs in the BTN involve only transcription, they typically must have much slower dynamics than the controller. For example, the slowest dynamic in Figure 1B is the synthesis of the HS operon from the plant in Figure 1A. Indeed, the rest of the controller is implemented entirely in the relatively faster PPI and σ^{32} translation, while transcription of *rpoH* is not regulated and so does not contribute to the dynamics of the controlled system [4].

The Implications of Structural Stability

The lack of structural stability in full systems of plant and controller may lead to the speculation that they are not robust. In fact, all complex, controlled systems lack structural stability when viewed at the full system level with controller dynamics included. A complete answer to this apparent paradox is a large subject in its own right, but some aspects are easily explained. One basic point is that the signs—whether interactions are activating (positive) or inhibiting (negative)—in most biological and technological networks must be fine-tuned, but once the signs of constants are appropriately fixed, their absolute values can often vary substantially with little effect. The number of different sign combinations in n constants grows exponentially as 2^n; thus, one (fine-tuned) choice of signs becomes a vanishingly small fraction of the total number of possibilities in any sufficiently large network. In other words, if signs are important, and they are in control systems, the resulting network cannot be structurally stable. It is also true that in both technology and biology it is much easier to manufacture components with robustly fixed signs than with precise absolute values.

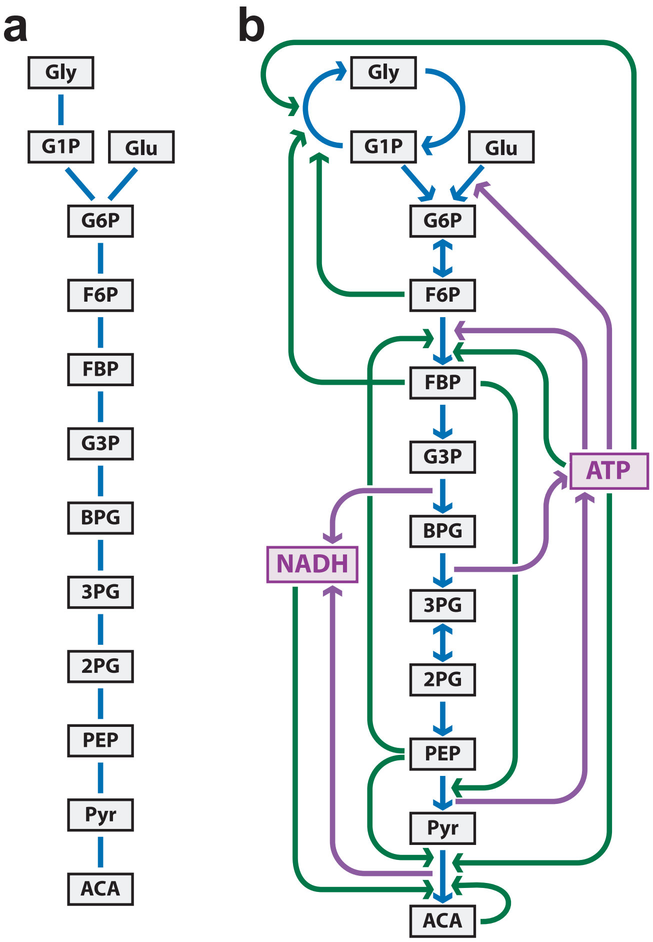

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Figure 3. Cartoons of a Generic Glycolysis Network in Bacteria

(A) A simple graph showing the main metabolites of glycolysis and their relationships.

(B) The same metabolites but also including reactions (solid blue lines), autocatalytic feedbacks (solid purple lines), and regulatory feedbacks (dotted black lines).

Thus, while structural stability as defined in Prill et al. may be too strong of a notion to be helpful in distinguishing between different control systems, this very strength further underscores the significance of the authors' results. All the plant motifs in the BTN, indeed, the entire BTN plant itself, are not merely stable, but have extremely strong structural stability. But the necessarily fine-tuned controller can stabilize these plants, and, furthermore, unstable plants are common in biology and technology, so the absence of unstable plants here still needs further explanation.

The Costs of Plant Instability

Perhaps the most relevant concept from control theory is that unstable plants are intrinsically more difficult to control than stable ones, and are generally avoided unless the instability confers some great functional advantage, which it often does [2,3]. A classic illustration of instability and control, the simple inverted pendulum experiment, can be easily tried at home, and illustrates the essential point without the mathematical details. Here the pendulum is the plant, and the human is the controller. The experiment can be done with sticks of different lengths or with an extendable pointer, holding the proximal tip between thumb and forefinger so that it is free to rotate but not otherwise slip. With the controlling hand fixed, this system has two equilibria, down and up, which are stable and unstable, respectively. By watching the distal tip and controlling hand motion, the up case can be stabilized if the stick is long enough. For an external disturbance, imagine that there is a virtual object making small motions in the vicinity of the distal tip, and your goal is to move the hand in such a way as to track this motion.

You will soon find that it is much easier to control the distal tip down than up, even though the components in both cases are the same. Because the up configuration is unstable, certain hand motions are not allowed because they produce large, unstable tip movements. This presents an obstacle in the space of dynamic hand movements that must be avoided, making control more difficult. If you make the stick shorter, it gets more unstable in the up case, evident in the short time it takes the uncontrolled stick to fall over. Shorter pendulums get harder and ultimately impossible to control in the up case, while length has little such effect on the down case. Also, the up stick cannot be stabilized for any length if only the proximal tip is watched, so the specific sensor location is crucial as well. This exercise is a classical demonstration of the principle that the more unstable a system the harder it is to control robustly, and control theory has formally quantified this effect in several ways (Text S1).

With this general context, a plausible conjecture is that the stability of the slow dynamics in the BTN plant is there, in part, to make control easier. It is the controller in Figure 1B that must be robust to the plant and disturbance in Figure 1A, not the other way around. Yet as the pendulum example illustrates, the stability of the plant can have a large impact on the achievable robustness of the controller. The BTN plant stability could additionally be a consequence of evolutionary constraints, in that the slow dynamics may have existed first (such as Figure 1A), and control was later layered (as in Figure 1B). If the slow dynamics are simply vestiges of an original, uncontrolled, and structurally stable network, their preservation, even by accident, still facilitates the full system level control. Perhaps such preservation could be the result of selective pressure on this system for robustness.

A Place for the Unstable Plant

While unstable plants are difficult to control, they are used when function requires it. Modern rockets are unstable in a manner similar to the up pendulum, and must be stabilized by active control systems. Toy rockets or fireworks without active control create stable plants by using large fins or tails that passively move the centers of pressure and gravity to make the dynamics more like the down pendulum, but at the expense of greater drag. Technology has abundant examples where similar efficiency and performance trade-offs lead to unstable plants with actively stabilizing controllers. Even bacteria have systems with unstable components that are nevertheless combined into feedback systems that are stable and robust.

Chemotaxis, cell movement toward a chemical attractant, is an example whereby an uncontrolled plant consisting of only cell and flagella would move essentially randomly, and, thus, would not be stable in any conventional sense. Yet with the full signal transduction system in place, the controlled runs and tumbles are biased to create effective chemotaxis, apparently using strategies common in engineering [8]. Glycolysis is often drawn as a "molecular motif" as in Figure 3A, without loops and therefore structurally stable, and the relationship between Figure 3A and the larger controlled Figure 3B is even more subtle than between Figure 1A and 1B. Figure 3B shows both positive autocatalytic feedback of ATP needed to fuel glycolysis and negative regulatory feedback, a combination that when sufficiently perturbed can lead to well-known instabilities, even with the control system intact [9]. These complex features of HS, chemotaxis, and glycolysis may not be accidental, but may be necessary consequences of unavoidable trade-offs, and as briefly sketched here, control theory supports this notion. Perhaps more persuasive is that these are three of the most thoroughly studied small networks in biology, and apparently no one has found alternatives, even theoretically, that convincingly improve on the efficiency and robustness of the wild type networks.

BTN motifs exhibit an extremely strong version of structural stability. Yet because of the organization involving both plant and controller, this apparently severe restriction on the BTN plants does not necessarily create a correspondingly severe constraint on function. For example, basic function of the plant motif in Figure 1A of manufacturing HS proteins is simple enough that only a minimal network is needed. Most of the network complexity is in the controller in Figure 1B, and to maximize the speed of the HS response, it is important to minimize the effects of any additional transcriptional events, which implies the plant must be kept simple. The result is that the BTN network as a whole is very flat with few long paths, which we conjecture by analogy to HS, allows the controlled system to have rapid response [10]. Perhaps a constructive next step would be to systematically contrast the strongly stable BTN plants with the less stable plants in chemotaxis and glycolysis. From an engineering perspective, all of these well-studied bacterial networks appear highly efficient and robust, tolerating trade-offs to achieve this well-engineered state [11]. And now, Prill et al. put at least one feature of bacterial transcriptional network motifs, their structural stability, into a much broader context. ■

Supporting Information

Text S1. Supplementary Notes: Elementary Feedback Concepts

Found at DOI: 10.1371/journal.pbio.0030392.sd001 (154 KB PDF).

Text S2. Feedback Control Theory

Found at DOI 10.1371/journal.pbio.0030392.sd002 (4.2 MB PDF).

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Supplementary Notes: Elementary Feedback Concepts

John C. Doyle, Marie E. Csete

Abstract: Convergent evolution in both biology and technology produces modular architectures with elaborate hierarchies of protocols and layers of feedback regulation, driven by demand for robustness to uncertain environments, while using often-imprecise components. This tutorial reviews the most elementary and well-known results from control theory of relevance to understanding feedback in biological networks.

A key starting point in developing a conceptual and theoretical bridge between engineering and biology is robustness, the preservation of particular characteristics despite uncertainty in components or the environment (*1*,*2*). Biologists and biophysicists new to studying complex networks often express surprise at a biological network’s apparent robustness (*3*). They find "perfect adaptation" and homeostatic regulation are robust properties of networks (*4*,*5*), despite "exploratory mechanisms" that can seem gratuitously uncertain (*6*,*7*,*8*). Some even conclude that these mechanisms and their resulting features seem absent in engineering (*8*,*9*). However, ironically, it is in the nature of robustness and complexity where biology and advanced engineering are *most* alike (*10*). Good design in both cases (e.g. cells and bodies, cars and planes) means users are largely unaware of hidden complexities, except through system failures. Furthermore, the robustness and fragility features of complex systems are shared *and* necessary. Although the need for universal principles of complexity and corresponding mathematical tools is widely recognized (*11*), sharp differences arise as to what is fundamental about complexity and what mathematics is needed (*12*). This tutorial is based on (*2*) and presents the most elementary aspects of well-known results in control theory.

Protocols are the most important aspect of modularity, and the most complex and critical protocols are for feedback control and the sensing, computing, communication, and actuation that implement it. Feedback control is both a powerful and dangerous strategy for creating robustness to external disturbances and internal component variations. Properly balanced it delivers such a huge benefit that both engineers and evolution capitalize extensively on feedback to build and support complex systems. Detailed elaboration of the nature of regulatory feedback is well beyond the scope of this tutorial, but an elementary “toy” model illustrates the *necessity* both of feedback to the function of complex systems and its “conservation of fragility” law. This is arguably the most critical and rigorously established robustness tradeoff in complex systems.

In most technologies as well as biochemistry it is relatively easy to build either uncertain, high gain components, or precise, low gain ones, but the precise, high gain systems essential to both biology and technology are impossible or prohibitively expensive to make, except using feedback strategies such as in Fig. 1. The simplest case to analyze is steady state gain, where after some transient, r and d are held constant, and y too approaches a constant $y=Rr+Sd$ (*13*). Solving $y=d+ACy+Ar$ gives

$$y = ASr + Sd = \frac{1}{C}\left(S-1\right)r + Sd$$

$$S \triangleq \frac{1}{1-F} \qquad F \triangleq AC$$

Ideally, perfect control would have $|S| = 0$, since that gives $y = -r/C$ ($R = -1/C$) completely independent of arbitrary variations in A and d. This can be achieved asymptotically if $A \to \infty$ and $-1/C >> 1$ then $F \to -\infty$, $|S| \to 0$, and $y \to r/C$. Then R amplifies r and is perfectly robust to external disturbance d and to variations in A (*14*). Choosing C small and precise, with A sufficiently large and even sloppy, is one effective, efficient, and robust way to make y a high gain function of r. $|S|$ measures the deviation from perfect control, and feedback can attenuate or greatly amplify the effects of uncertainties. Defining *fragility* as $\log|S|$, note that $F < 0$ iff $|S| < 1$ iff $\log|S| < 0$. $F > 0$ makes $\log|S| > 0$, amplifying d and uncertainty in A, and $F \to 1$ makes $\log|S| \to \infty$ (*15*).

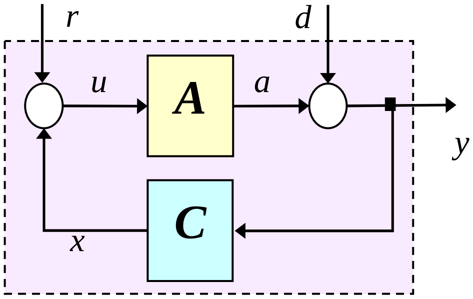

Figure 1. Minimal feedback system with actuator *A* and controller/sensor *C*. Goal is for response *y* to amplify reference *r,* independent of external disturbance *d,* and variations in *A.*

Dynamics

This story is incomplete and even misleading without dynamics. The simplest possibility is for *A* and *C* to be 1st-order differential equations

$$\begin{aligned} C &: x' = -k_1 y - k_2 x & y &= d + a \\ A &: a' = gu & u &= r + x \end{aligned} \tag{1}$$

C is a low pass filter with internal state *x* and parameters k_1 and k_2. *A* is a pure integrator with state *a* and gain *g* (*16*), and we'll assume that $g > 0$. This system can be written in "state space" form as

$$\begin{bmatrix} x' \\ a' \end{bmatrix} = \begin{bmatrix} -k_2 & -k_1 \\ g & 0 \end{bmatrix} \begin{bmatrix} x \\ a \end{bmatrix} + \begin{bmatrix} -k_1 d \\ gr \end{bmatrix} \tag{2}$$

Stability depends on the eigenvalues λ_j of the matrix

$$\begin{bmatrix} -k_2 & -k_1 \\ g & 0 \end{bmatrix}$$

which are the zeros of the characteristic polynomial

$$s^2 + k_2 s + g k_1$$

having negative real part ($\mathrm{Re}\left(\lambda_j\right) < 0$). It is easily checked that this holds and the system is stable iff $gk_1>0$ and $k_2 > 0$, or equivalently $k_1 > 0$ and $k_2 > 0$. Stability here means the solution in the states *x* and *a* converges to the origin for all initial conditions, and any bounded input in *r* and/or *d* gives bounded state. For this system these two notions are equivalent, but in general there are a variety of notions of stability.

Thus given $g > 0$, stability depends only on the signs of k_1 and k_2, and holds if and only if both are positive. Since there are only two constants, this means that ¼ of the space of values is stable, but then it is stable for all positive values. Thus stability is not robust to sign changes, but with fixed signs is very robust to magnitude changes. This is a typical situation and has apparently been a large source of confusion regarding the robustness of biological networks. For *n* constant, the number of different sign combinations grows exponentially as 2^n and thus one (fine-tuned) choice of signs becomes a vanishingly small fraction of the total number of possibilities in any sufficiently large network. For example, a characteristic polynomial such as

$$s^n + k_n s^{n-1} + \cdots + k_2 s + k_1$$

has roots with negative real part only if all k_j are positive, although additional (polynomial) constraints are needed to give if and only if conditions. In other words, if signs are important, and they are in control systems, the resulting network cannot be structurally stable. It is also true that in both technology and biology it is much easier to manufacture components with robustly fixed signs than with precise absolute values, so this is not necessarily a stringent constraint on control systems. Much more important is that this type of stability is not as important as the more severe constraint of robust transient response.

This type of control is called "integral feedback." The parameters *g*, k_1, and k_2 might typically be functions of underlying physical quantities such as temperature, binding affinities, concentrations etc. and thus might vary widely. The response $y(t)$ to steps in *r* and *d* are shown in Figure 2 over two orders of magnitude in *g* and $k_1 > 0$.

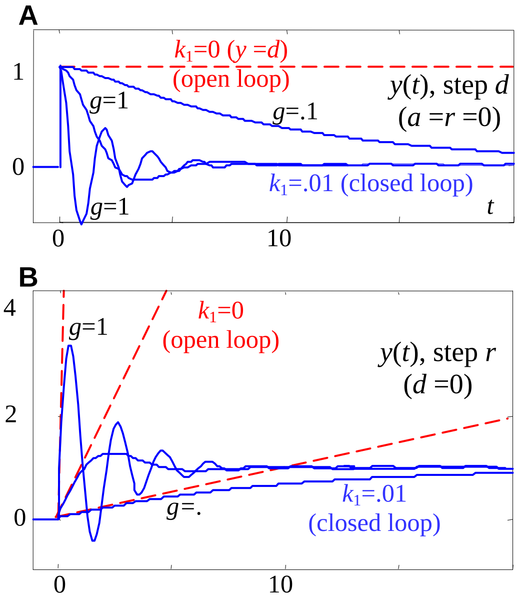

Figure 2. Closed (k_1=0, blue) vs. open (k_1=.01, red) loop response *y*(*t*) to step changes at *t*=0 in **(A)** *d*(*t*) (*r*=0) and **(B)** *r*(*t*) (d=0) for *g*=.1, 1, 10, k_1=.01, k_2=10 k_1. Note the extreme divergence (k_1=0) vs. convergence (k_1=.01) as t→∞.

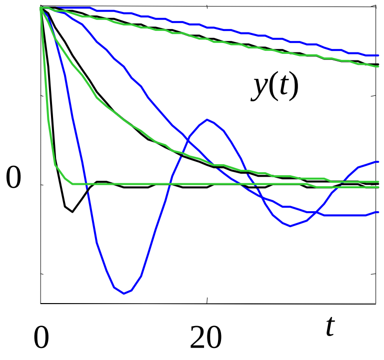

Figure 3. Zoom of Figure 2A with k_1=.01 (blue), .1 (black), 1 (green), and k_2=10k_1 added for each value of *g*.

This simple protocol of *integral feedback* produces extremely robust external behavior even from wildly varying components (the blue solid versus red dashed lines in Figure 2b) and converges to the steady state $y=(k_2/k_1)r$ independently of *arbitrarily* large variations in gain g and disturbance d (*17*). If $k_2>>k_1$, $y=(k_2/k_1)r$ is a high gain amplifier as well (*18*). The individual values of g, k_1 and k_2 influence the rate of convergence to steady state, but only (k_2/k_1) determines its value. Thus robust high steady state gain can be achieved with uncertain and small parameters with the right feedback protocol. Figure 3 shows that variations in both g and k_2 of orders of magnitude have modest impact, and only on early transient behavior.

The *protocol* here is the structure of the equations, including the integral feedback and the signs of the parameters. Modules are the implementations of the actuator and controller. As with the Lego example in (*2*), this protocol must be “fine-tuned” (since rewiring components or flipping signs typically creates exponentially growing instabilities), but this allows the modules to vary widely with minimal effect (*19*). Integral feedback is used ubiquitously in engineering (*20*) and is likely to be ubiquitous in biology as well, to achieve everything from homeostatic regulation to "perfect adaptation," and preliminary investigations confirm this impression (*21*,*22*,*23*). One reason is that integral feedback is both sufficient *and necessary* for perfect and robust steady state tracking. Intuitively, necessity follows from the fact that in steady state, $a=y-d$ must perfectly cancel *any* constant (step in) d, while the input u to A cannot depend on this d, since y does not. Thus, A (or C) must contain an *internal model* of the *dynamics* of d, which for step changes is a pure integrator (*24*), which produces unbounded outputs to constant inputs. Thus, open-loop hypersensitivity is necessary for closed loop robustness, and the behavior in Figures 2 and 3 is not an accident.

Conservation laws

Fragility also enters in the transient response. When g is increased, the response is faster but oscillatory (Figure 2). Figure 4 plots fragility $\log|S(\omega)|$ versus ω where $Y(\omega)$ and $D(\omega)$ are Fourier

$$\log|S(\omega)| = \log\left|\frac{Y(\omega)}{D(\omega)}\right| \qquad (3)$$

transforms of y and d. For increasing g, low frequency robustness ($\log|S(\omega)|<0$) is improved but at the expense of increased fragility ($\log|S(\omega)|>0$) at higher frequencies (*25*). In fact, it can be proven that for all g

$$\int_0^\infty \log|S(\omega)|\,d\omega = 0 \qquad (4)$$

so net fragility is, in this sense, a conserved quantity.

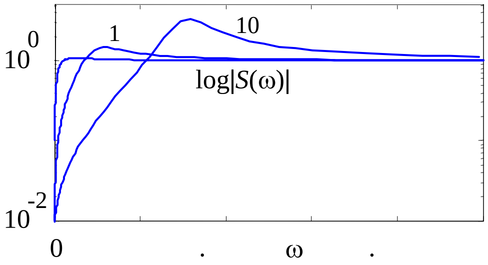

Figure 4. log**|***S*(ω)**| vs.** ω for responses in 2A). The peaks in log**|***S*(ω)**|** correspond to the oscillations in 2A) and 2B). Note the equal areas under the curves for log**|***S*(ω)**|.**

Robustness ($\log|S(\omega)|<0$) is paid for by an equal fragility ($\log|S(\omega)|>0$) which amplifies d and uncertainty in A (*26*). This quite general result also holds for arbitrary parameters, control systems, and disturbances (*27*). Thus there are always nonconstant (e.g. sinusoidal) $d(t)$ that would be *amplified* in $y(t)$. Such d could be perfectly rejected too, but only by adding internal models as *complex as the external environment* that generates d. While such modeling is only possible for simple idealized laboratory environments, even approximate attempts can drive an extreme complexity spiral in real systems, and *any* controller is still subject to the constraint in eq. (4). The key to good control design then is insuring that this fragility is tolerable, and occurs where uncertainties are relatively small.

Even these simple toy examples show the robust yet fragile features of complex regulatory networks. Their outward signatures are, ironically, extremely constant regulated variables *yet* occasional cryptic fluctuations. They have extraordinary robustness to component variations *yet* rare but catastrophic cascading failures. These apparently paradoxical combinations can easily be a source of confusion to experimentalists, clinicians, and theoreticians alike (*28*), but are intrinsic features of highly optimized feedback regulation. Since net robustness and fragility are constrained quantities, they must be manipulated and controlled with and within complex networks, even more than energy and materials. Figure 2b shows how extreme open vs. closed loop behavior can be, and thus how dangerous loss of control is to a system relying on it. The tradeoff in equation (4) shows that even when working perfectly, net fragility is constrained, and thus some transient amplification is unavoidable.

The *necessity* of integral feedback and the fragility constraint in equation (4) thus describe *laws*, not protocols, perhaps the two simplest such laws from control theory. Controllers that are more complex, with additional dynamics and multiple sensors and actuators, offer more refinement in performing robustness-fragility tradeoffs. Adding to regulatory complexity is also evolutionarily relatively easy. Faster components allow for faster closed-loop responses. All are used in both biology and engineering but all are still ultimately subject to equation (4). Control engineers must contend with this tradeoff, and its generalizations to more complex structures dominate control system design. It may be that such tradeoffs dominate and constrain evolution and biology as well.

The cost of instability

The simplest change that introduces plant instability is for A to be changed to

$$C: x' = -k_1 y - k_2 x \quad y = d + a$$

$$A: a' = \sigma a + gu \quad u = x$$

where for simplicity r has been eliminated and C is unchanged, and we'll continue to assume that $g > 0$ and also that $\sigma \geq 0$. With $k_1 = k_2 = 0$ there is no feedback, and the system is "open loop" with an unstable pole at σ. The state space form is

$$\begin{bmatrix} x' \\ a' \end{bmatrix} = \begin{bmatrix} -k_2 & -k_1 \\ g & \sigma \end{bmatrix} \begin{bmatrix} x \\ a \end{bmatrix} + \begin{bmatrix} -k_1 d \\ 0 \end{bmatrix}$$

Stability again depends on the eigenvalues of the matrix

$$\begin{bmatrix} -k_2 & -k_1 \\ g & \sigma \end{bmatrix}$$

or the zeros of the characteristic polynomial

$$s^2 + \left(k_2 - \sigma\right)s + \left(gk_1 - k_2\sigma\right)$$

so this system is asymptotically stable iff

$$k_2 > \sigma \qquad g\frac{k_1}{k_2} > \sigma$$

Thus the region of stability shrinks as σ gets larger. The more serious limitation is that equation (4) now becomes

$$\int_0^\infty \log\left|S(\omega)\right| d\omega \geq \pi\sigma \qquad (5)$$

so that the tradeoff in equation (4) is aggravated. While unstable plants are common in engineering and biology, they are typically used only when efficiency or performance demands it, and otherwise are avoided since unstable plants are intrinsically harder to control.

Implications for biology and engineering

Success of systems biology will certainly require modeling and simulation tools from engineering (*29,30*), where experience shows that brute force computational approaches are hopeless for complex systems involving protocols and feedback. Highly fragile features require highly sophisticated modeling, whereas robust features often have adequate models that are greatly simplified. For example, if Fig. 1 was for a module in a larger system, the steady-state gain $y=(k_2/k_1)r$ depends only on (k_2/k_1) and no other parameters, potentially simplifying experiments and modeling. If transient dynamics or component failure were of interest, more details would be needed, determined more by the rest of the system than by the internal components.

Many challenges of post-genomic biology are converging to those facing engineers building complex networks and "systems of systems." Engineering theory and practice are now undergoing a revolution as radical as biology's. The simple ideas here only hint at the possibilities. For example, more complex control protocols than Figure 1, used in both engineering and biology, can ameliorate though not eliminate the constraint in (4), but sophisticated theory is needed to elucidate the issues. Realistic models of biological networks will not be simple, with multiple feedback signals, nonlinear component dynamics, numerous uncertain parameters, stochastic noise models (*31*), parasitic dynamics, and other uncertainty models. Scaling to deal with large networks will be a major challenge. Fortunately, researchers in robust control theory, dynamical systems, and related areas have been vigorously pursuing mathematics and software tools to address exactly these issues and apply them to complex engineering systems (*32,33*). Biological applications are new, but progress so far is encouraging.

Experiments, modeling and simulation, and theory all have fragilities, but they are complementary, and through the right protocols, have the potential to create a robust "closed-loop" systems biology. Biologist's frustrating experience with theory has been primarily in an open-loop mode, where simple and attractive ideas can be wrong but receive enormous attention. Biology is the only science where feedback control and protocols play a dominant role, so it should not be surprising that there would be popular theories, coming from within science, which did not emphasize these issues. Biologists and engineers now have enough examples of complex systems that they can close the loop and eliminate specious theories (33). For example, Internet technology is rich in protocols and feedback, and a deep, rigorous, and practically relevant theory has recently been developing. Even though it is poorly understood by nonexperts and has become a focus of many specious theories, details and enormous data sets are available, and it makes an attractive example to compare with biological networks(see additional references).

Notes

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1. Complex systems may be nonlinear, heterogeneous, large-scale, hierarchical, adaptive etc. but these are subsidiary issues here.
2. Csete M, Doyle J, Reverse Engineering of Biological Complexity, *Science* 295 (5560): 1664-1669 Mar 1 2002
3. Fragility here has the specific meaning of large and deleterious changes in particular system properties due to possibly small but specific variations in the environment or in components. Robustness is often used more broadly, but here is roughly the inverse of fragility. Their subtle interplay is the point of this paper.
4. Alon, U., Surette, M.G., Barkai, N., Leibler, S. *Nature* 397, 168-171 (1999).
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7. E.g. chemotaxis, immune response, microtubule formation, angiogenesis, neurogenesis, and evolution itself. See (*6*)
8. Kirschner, M., Gerhart, J., Mitchison, T. *Cell* 100, 79-88 (2000).
9. Chicurel, M. *Nature* 408, 900-901 (2000).
10. This has a long history, see for example Wiener, N. *Cybernetics*, (New York, Wiley, 1948)
11. "Complex Systems" Special Issue, *Science*, V 284, No. 5411 (1999)
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13. Steady state here means simply that all variables in Figure 2 (r, d, y, A, C, etc) approach constants, which can be solved for algebraically.
14. ">>" means "very much greater than."
15. An important use of positive feedback is to *deliberately* destabilize equilibria and amplify small differences to create switches and break symmetries and homogeneities. This can create patterns then maintained using negative feedback. Positive feedback is also critical to autocatalysis in growth and metabolism.
16. $a'=gu$ means a (the output of A) is a time integral of gu, where u is the input to A.
17. Stability is easily shown using standard methods of linear systems. Steady state values can be found (in a stable system) by setting all time derivatives to 0, yielding $gk_1y=gk_2r$ or $y=(k_2/k_1)r$.
18. Mechanisms often exist that allow controller parameters (e.g. k_1 and k_2) to be much less uncertain than g and d. It is often even easier to make *ratios* such as (k_2/k_1) largely invariant to variations in underlying physical quantities affecting the individual k_1 and k_2.
19. Except (k_2/k_1) if precise gain is desired.
20. The national power grid has integral control at the >3,000 power plants to regulate frequency and voltages of delivered power, oil refineries have >10,000 such control loops, and Internet congestion control involves a form implemented as part of TCP (Transport control protocol). See additional references for more details, proofs, and examples.
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22. Levchenko, A., Iglesias, P. A. *Biophys. J.* 82, 50-63 (2002).
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24. This argument can be made rigorous and is a standard elementary result in control theory. It is a special case of the internal model principle.
25. For sufficiently large g the frequency domain peak and time domain transients become unacceptably large, though still stable.
26. One interpretation is that negative feedback is always balanced by an equal and opposite positive feedback. Strictly speaking, with dynamics this is not well defined, and $\log|S(\omega)|$ gives the correct generalization.
27. Bode, H.W., *Network Analysis and Feedback Amplifier Design* (1945). Relatively rare circumstances can involve an inequality (≥). This is worse, but means (4) is an inequality constraint rather than a pure "conservation" law.
28. The robust yet fragile nature of highly optimized complex regulatory networks can be mistakenly attributed to various kinds of bifurcations and "order-disorder" transitions (e.g. phase transitions, critical phenomena, "edge-of-chaos," pattern formation, scale-free, etc.).
29. E.g. the Boeing 777 alone required a global software and computing infrastructure with roughly ten thousand workstations, terabytes of data, and a billion dollar price tag.
30. www.sbml.org
31. Gillespie, D.T. *J. Chem. Phys*. 113, 297-306 (2000).
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