Molecular Cell
Review

The Utility of Paradoxical Components in Biological Circuits

Yuval Hart[1] and Uri Alon[1,*]
[1]Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel
*Correspondence: uri.alon@weizmann.ac.il
http://dx.doi.org/10.1016/j.molcel.2013.01.004

A recurring theme in biological circuits is the existence of components that are antagonistically bifunctional, in the sense that they simultaneously have two opposing effects on the same target or biological process. Examples include bifunctional enzymes that carry out two opposing reactions such as phosphorylating and dephosphorylating the same target, regulators that activate and also repress a gene in circuits called incoherent feedforward loops, and cytokines that signal immune cells to both proliferate and die. Such components are termed "paradoxical", and in this review we discuss how they can provide useful features to cell circuits that are otherwise difficult to achieve. In particular, we summarize how paradoxical components can provide robustness, generate temporal pulses, and provide fold-change detection, in which circuits respond to relative rather than absolute changes in signals.

Introduction

A biological circuit is a collection of interacting components that carry out a function (McAdams and Shapiro, 1995; Rao and Arkin, 2001; Alon, 2007). A "paradoxical component" is defined as a component of the circuit that simultaneously has two opposite effects on the same target or biological process (Stadtman and Chock, 1977; Lenardo, 1991; Chastain and Chollet, 2003; Hunter, 2005; Hart et al., 2011a, 2012) (Figure 1A). This feature can also be called antagonistic pleiotropy or antagonistic bifunctionality. Examples are bifunctional enzymes that carry out two opposing reactions, regulators that activate and also repress a gene, and cytokines that signal a cell to both proliferate and die. It should be noted that paradoxical components differ from negative feedback, in which a component's action causes a change in its own activity (Figure 1B). In this review, we synthesize work from a wide range of fields and ask what functional benefits a circuit might achieve by using a paradoxical component rather than placing the two opposing functions in different components. We describe circuits in systems ranging from gene regulation and metabolism to development and immunology. We emphasize that paradoxical components can provide cell circuits with useful features that cannot be easily achieved otherwise.

The best-studied feature provided by paradoxical components is robustness: allowing the circuit to function precisely and accurately provide a desired input-output relationship despite naturally occurring variations in the concentrations of its components. Other features that can be provided by paradoxical components, in cases where they act on two different timescales, are the generation of robust pulses and the detection of relative (rather than absolute) changes in signal. We aim here, in each case, to highlight open areas for future research.

Bifunctional Enzymes that Catalyze Antagonistic Reactions

All enzymes catalyze a reaction and its reverse, with the net flux determined by the concentrations of substrate and product. Paradoxical enzymes are defined as a different and more specific phenomenon: bifunctional enzymes that catalyze two different reactions, often by means of two different catalytic sites, such that the two reactions are antagonistic. An example is a bifunctional enzyme that is both a kinase and a phosphatase for the same target. What might be the use of such contradictory reactions by the same protein?

An example of such antagonistic bifunctionality occurs in bacterial two-component systems. A canonical example is the osmotic response system EnvZ-OmpR. This system has a receptor EnvZ, which we will call "X", and a messenger protein OmpR, which we will call "Y". The receptor X phosphorylates the messenger Y, forming Y-P, which is a transcription factor of osmo-response genes (more precisely, X is an autokinase and a phosphotransferase, using ATP to phosphorylate itself and then transferring the phosphoryl to Y). Silhavy and colleagues discovered that X carries out two antagonistic reactions (Hsing et al., 1998): it not only acts as a kinase for Y; it is also the phosphatase of Y-P (Figures 2A and 2B) (Goldberg et al., 2010; Capra and Laub, 2012).

This bifunctionality, acting as both a kinase and phosphatase, was suggested by Russo and Silhavy (1993) to enable robustness in the circuit. This was modeled mathematically and demonstrated experimentally by Batchelor and Goulian (2003): bifunctionality makes the input-output relation of the system robust with respect to fluctuations in the levels of the Y protein. In other words, the amount of Y-P at a given level of input signal (e.g., osmolarity of the medium) is insensitive to changes in the amount of total Y protein.

Shinar et al. (2007) extended the theoretical analysis of this system, providing an analytical solution for its behavior. The special features of the receptor—autokinase, phosphotransferase, and phosphatase—combine to make Y-P levels insensitive to variations in the concentrations of all components—X and Y—and yet responsive to the input signal of the system. The intuitive reason is that an increase in the concentration of any component increases both phosphorylation and dephosphorylation

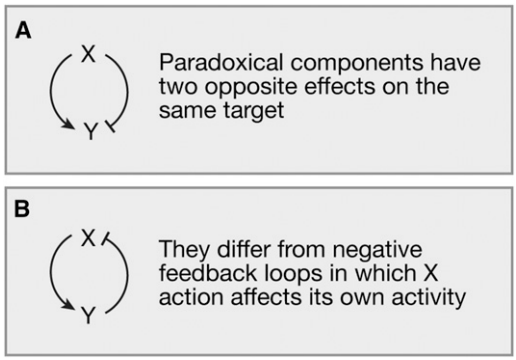

Figure 1. Paradoxical Components Have Two Simultaneous Opposing Effects on the Same Target

(A) A paradoxical component can be symbolized using a positive- and negative-interaction arrow from the component to its target.

(B) Paradoxical components should be distinguished from feedback loops, in which a component's activity affects itself. Often, paradoxical components can also participate in feedback loops.

rates by the same factor (Russo and Silhavy, 1993), thus canceling out the effect on the steady-state output Y-P (Figure 2B).

A more standard design for a signaling system, wherein receptor X acts as a kinase and a separate protein Z as a phosphatase, would make the output Y-P depend on the concentrations of all proteins in the circuit (Figure 2A). Thus, the bifunctional nature of the two-component receptor seems to provide the signaling system with robustness to naturally occurring fluctuations in its component concentrations. This bifunctional design is thought to occur in the vast majority of bacterial two-component systems (Capra and Laub, 2012).

The theme of robustness based on paradoxically bifunctional enzymes has been extended to several other systems. In each system, antagonistic bifunctionality is suggested to provide a robust input-output relationship, but each system studied so far has also shown a different combination of features that generate this robustness. One example is in the nitrogen-assimilation system of *E. coli*. The key enzyme that assimilates ammonia into biomass is the dodecameric enzyme GlnA, which produces the amino acid glutamine, Q. The dilemma is that Q is made at the expense of a carbon backbone that is a key metabolite in the tricarboxylic acid (TCA) cycle, alpha-ketoglutarate, denoted K. Making too much Q depletes K; therefore, the Q/K ratio is very important and stays about constant in a wide range of conditions (Senior, 1975; Brauer et al., 2006).

The robustness of the Q/K ratio depends on a bifunctional enzyme AT/AR which both activates and deactivates GlnA by removing and adding an adenylyl modification. The twist is that AT/AR may bind two GlnA subunits in the same dodecamer, and hence shows a strong avidity effect: if it binds one subunit, it is likely to bind both. Thus, a ternary complex T in which the bifunctional enzyme binds two substrates, one modified and the other unmodified, carries out most of the reactions (Figure 2C). The rates of adenylation and deadenylation are equal at steady state: $v_1(Q,K)\ T = v_2(Q,K)\ T$. Here, the specific rates of the two reactions carried out by the bifunctional enzyme are v_1 and v_2, and both depend on Q and K. The ternary complex cancels out, and thus steady state requires $v_1(Q,K) = v_2(Q,K)$. This can only result by the Q levels changing until a fixed relation between Q and K is reached (a fixed Q/K ratio). This mechanism was tested experimentally using mass spectrometry, showing that bifunctionality is required for the robustness of the Q/K ratio and the robustness of the growth rate in the face of large, experimentally induced variations in GlnA and AT/AR levels (Hart et al., 2011a) (Figure 2C).

Robustness mechanisms have been proposed for other antagonistically bifunctional enzymes: the enzyme IDHKP that regulates isocitrate dehydrogenase at the gate of the glyoxylate bypass in the bacterial TCA cycle (LaPorte et al., 1985; Shinar et al., 2009; Dexter and Gunawardena, 2012) and the enzyme that regulates a key step (catalyzed by PPDK) in the carbon-fixation pathway of C_4 plants (Hart et al., 2011b).

Bifunctional control also occurs in the glycolysis regulator PFK2 in mammalian metabolism (Pilkis et al., 1983), where it has been suggested to implement coherent switching in tissues such as those of the liver (Xu and Gunawardena, 2012). Intriguingly, central regulatory small molecules in bacteria, ppGpp and cyclic diguanine, are made and degraded, in part, by bifunctional enzymes (Dennis et al., 2004; Hengge, 2009); these systems have not yet been explored in terms of their robustness. The theoretical analysis of antagonistically bifunctional enzymes has led to a powerful mathematical theorem that can predict which components of a complicated biochemical reaction system might be robust (Shinar and Feinberg, 2010; Karp et al., 2012).

It is possible that many more antagonistically bifunctional enzymes exist than are currently known; in principle, the same effects can also be produced by distinct monofunctional enzymes that work together as a complex to carry out opposing reactions (Figure 2D). One possible example is the RhoA enzyme in cytokinesis, in which a GTP exchange factor and its antagonist, a GTPase-activating protein, work primarily when complexed together (Mishima et al., 2002; Somers and Saint, 2003; Yüce et al., 2005; Miller and Bement, 2009). Additional examples might occur in signaling systems with scaffolds that hold together enzymes with antagonistic roles. These possibilities offer exciting avenues for research.

Bifunctional Inhibitors in Developmental Pattern Formation

We now turn to protein circuits that carry out the patterning of tissues in development (Slack, 1991; Davidson, 2010). Initial pattern formation in embryos is carried out, in many cases, by gradients of morphogens. The gradients are formed by the diffusion of morphogen molecules away from their source. Explaining the robustness of the patterns is a challenge; making too little or too much morphogen at its source would mean, in the simplest models, a narrower or wider gradient and thus distorted patterns.

Naama Barkai, Ben-Zion Shilo, and colleagues discovered principles of protein circuits that provide robust pattern formation in a series of elegant studies (Ben-Zvi et al., 2008, 2011; Eldar et al., 2002; Haskel-Ittah et al., 2012). At the core of robust mechanisms are paradoxical roles of certain molecules. In particular, an inhibitor of the morphogen—a protein that prevents the morphogen from signaling to cells—was found to have a second role: enhancing the diffusion constant of the morphogen and thereby helping it to diffuse farther and increase

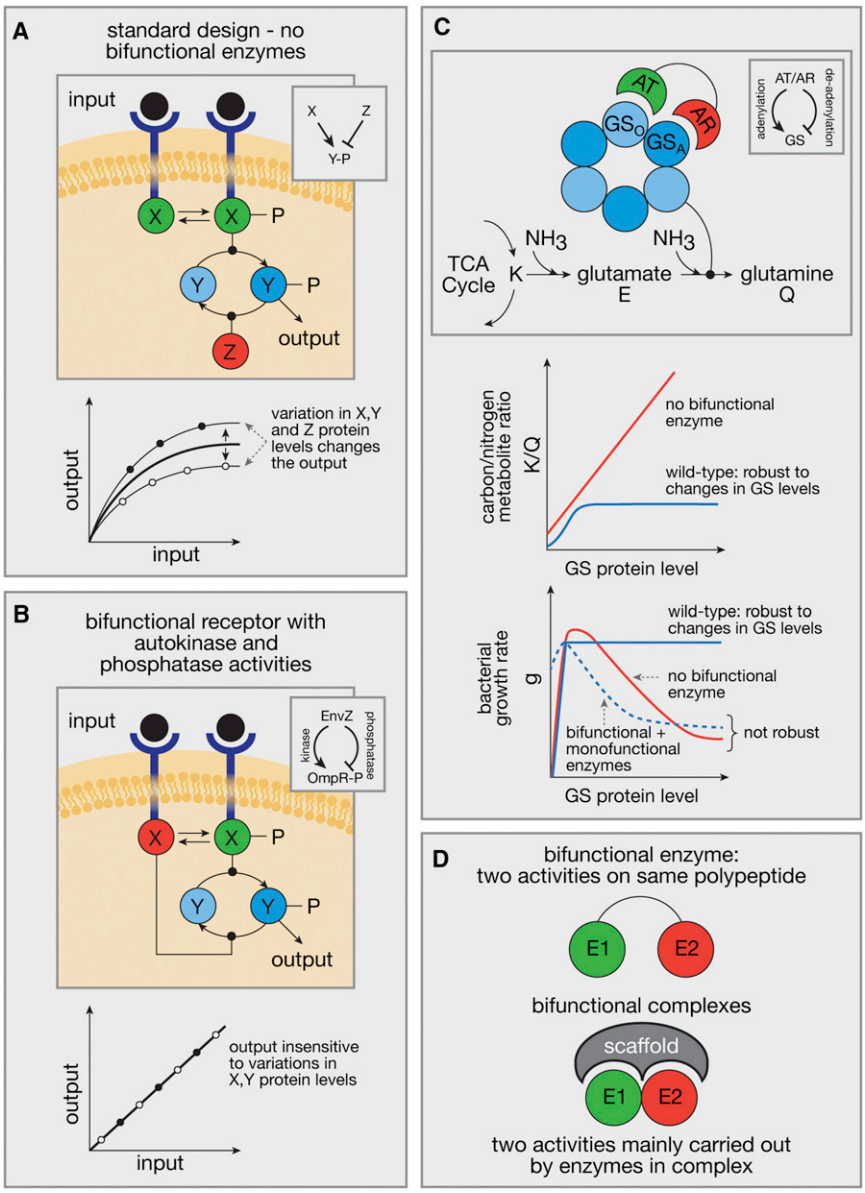

Figure 2. Bifunctional Enzymes Can Provide a Robust Input-Output Relationship, in which Output Depends on Input Signal, but Not on the Concentration of Any of the Proteins in the Circuit

(A) A monofunctional model of a two-component signaling system. Two separate enzymes, X and Z, phosphorylate and dephosphorylate Y, respectively. In this model, output levels (phosphorylated Y, denoted Y-P) are sensitive to changes in X, Y, and Z levels.

(B) Most bacterial two-component systems have a bifunctional receptor—an autokinase and phosphotransferase that phosphorylates Y, and also acts as a phosphatase that dephosphorylates Y-P. Output levels are insensitive to changes in X or Y levels and depend only on kinetic rate constants.

(C) In the nitrogen assimilation system of *E. coli*, a bifunctional enzyme, AT/AR, both adds and removes adenylyl groups from the dodecameric enzyme glutamine synthetase (GS). The active form of GS is unadenylated, GS_0. GS assimilates NH_3 to form glutamine, Q, whose carbon backbone is the TCA intermediate alpha-ketoglutarate, K. In *E. coli*, the Q/K ratio is insensitive to large changes in GS levels. Without the bifunctional enzyme, the Q/K ratio is highly sensitive to GS levels, rising steeply as GS increases. Robustness is evident also in the bacterial growth rate, where experiments show that removal of the bifunctional enzyme or expression of a monofunctional enzyme mutant in the presence of the native system abolishes the robustness of the growth rate to changes in GS levels (Hart et al., 2011a).

(D) Bifunctionality can in principle also be attained by two distinct antagonistic enzymes that are activated only when jointly bound to a scaffold, forming a bifunctional complex.

its range. Furthermore, the inhibitor is degraded when bound to the morphogen, but not when unbound. Together, these features combine to form a shuttling mechanism, which creates a robust gradient (Figure 3). The gradient is robust in the sense that its precise shape is unchanged despite variation in the production rates of all components of the system. Experimental tests of the shuttling mechanism have been presented in fruit flies and frogs (Eldar et al., 2002; Ben-Zvi et al., 2008; Haskel-Ittah et al., 2012). An extension of the shuttling mechanism with two bone morphogenetic protein (BMP) morphogen ligands with feedback repression can add an additional feature known as scaling: body proportions scale with the total organism size (Ben-Zvi et al., 2008, 2011; Ben-Zvi and Barkai, 2010).

An additional case of paradoxical components in tissue patterning may occur in notch-delta signaling. Delta has opposite effects on its target notch depending on whether the notch is in the same cell or in a neighboring cell (Sprinzak et al., 2010, 2011). This design helps make sharp, short-range patterns possible.

Incoherent Feedforward Loops

Two opposing effects can also be carried out by transcription regulators. This is found in a very common network motif called the incoherent feedforward loop (IFFL) type 1 (I1-FFL) (Milo et al., 2002; Shen-Orr et al., 2002; Alon, 2007; Shoval and Alon, 2010). The I1-FFL is a circuit in which a regulator, X, activates gene Z and also activates Y, a repressor or inhibitor of Z. Thus, X activates Z directly, but also inhibits it indirectly through Y (Figures 4A and 4B). This differs from a feedback loop, in which X affects its own activity (for example, a negative feedback loop in which X activates Y, which in turn inhibits X). For definitions of the other types of FFLs, see Mangan and Alon (2003).

The IFFL appears hundreds of times in bacteria (Milo et al., 2002; Eichenberger et al., 2004; Mangan et al., 2006) and in yeast (Lee et al., 2002; Milo et al., 2002). In animal cells, the I1-FFL is a major building block of circuits. For example, it appears numerous times in the transcriptional networks of embryonic

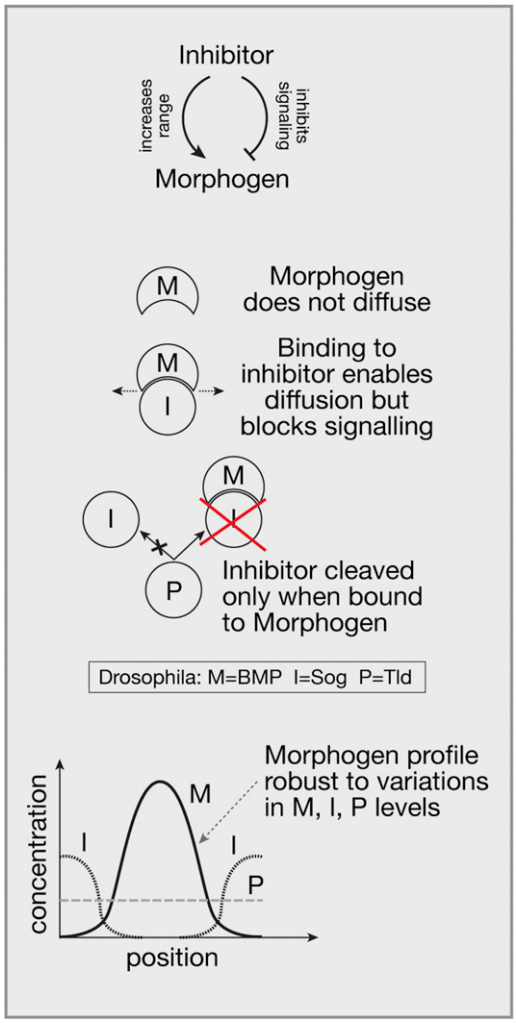

Figure 3. Bifunctional Inhibitors of a Morphogen Can Provide Robust Pattern Formation in Developmental Systems

An inhibitor (I) bound to a morphogen (M) prevents its signaling but enables its diffusion across the patterning field and thus increases its range. In addition, inhibitors are cleaved by the protease, P, only when bound to M. This shuttling mechanism produces a robust patterning insensitive to gene dosage or the concentrations of M, P, and I total protein (Eldar et al., 2002). An example in *Drosophila* embryo dorsal patterning is I = Sog, M = BMP, and P = Tld.

stem cells (Boyer et al., 2005) and hematopoietic stem cells (Swiers et al., 2006), in innate immune regulation (Chevrier et al., 2011), downstream of the Notch signaling pathway (Krejcí et al., 2009), and in fly eye development (Johnston et al., 2011). A recent comprehensive analysis of human transcription networks identified numerous instances of the feedforward loop network motif (Gerstein et al., 2012).

The indirect arm of the IFFL has a built-in delay compared to the direct arm, because Y needs to be produced and to reach a concentration sufficient to inhibit Z. When Y is a transcription factor, this delay is substantial, on the order of a cell generation or the lifetime of Y, whichever is shorter. This delay is thus in the range of tens of minutes to hours. When Y is a microRNA (Hornstein and Shomron, 2006; Osella et al., 2011; Ebert and Sharp, 2012), the delay is probably much shorter, on the order of minutes.

IFFLs are organized into higher-level motifs in at least two ways: The same X and Y regulators can control multiple output genes, resulting in multioutput IFFLS (Kashtan et al., 2004). IFFLs can also be linked with other FFLs to form FFL cascades, as occurs in the sporulation subnetwork of *B. subtilis* (Eichenberger et al., 2004; Wang et al., 2006).

The first functions demonstrated for IFFLs were the speedup of gene-circuit response times and generation of pulses of expression (Basu et al., 2004; Mangan and Alon, 2003; Mangan et al., 2006; Alon, 2007; Shoval and Alon, 2010; Macía et al., 2009). IFFLs can act as a temporal derivative for time-varying inputs (Basu et al., 2004). Their parameter sensitivity and ability to filter out noise were analyzed (Tyson et al., 2003; Ma et al., 2009; Ghosh et al., 2005; Kittisopikul and Süel, 2010).

More recently, additional functions have been found experimentally. The first of these new functions is the generation of nonmonotonic input-output relations: the output Z is highest at an intermediate level of X (Figure 4C) (Entus et al., 2007; Kaplan et al., 2008; Kim et al., 2008). This biphasic response occurs when X activity needed to turn on Z is lower than that needed to turn on Y expression. A biphasic response has been demonstrated in the IFFL controlling galactose genes in *E. coli* (Kaplan et al., 2008) and in synthetic IFFLs (Entus et al., 2007).

Incoherent FFLs often employ small inhibitory RNAs, microRNAs, as the Y component, instead of transcription factors (Bleris et al., 2011; Ebert and Sharp, 2012) (Figure 4D). In this case, the delay can be short, on the order of minutes. As such, the circuit is unlikely to generate pulses, because pulses require a substantial delay between the two arms of the IFFL. It has been suggested that these microRNA IFFLs provide homeostasis to Z during changes in X activity. Such changes include global variations between different cells (Elowitz et al., 2002; Blake et al., 2003, 2006; Paulsson, 2004; Raser and O'Shea, 2005; Newman et al., 2006; Sigal et al., 2006), in nuclear volume, X concentration, or X activity. An increase in X activity generates an increase in Z, which is counteracted by a proportional increase in Y (Osella et al., 2011). An IFFL with a microRNA called mir7, within a larger circuit, was found to impart robustness to developmental pathways in the face of temperature fluctuations (Li et al., 2009). A synthetic IFFL in mammalian cells made output levels insensitive to the dose of transfected vector bearing all components of the circuit (Bleris et al., 2011) (Figure 4E).

An additional function that can be carried out by IFFLs is fold-change detection (FCD) (Goentoro et al., 2009; Shoval et al., 2010). In FCD, a circuit responds to (relative) fold changes in input signal, rather than to absolute changes. For example, consider a step-like input stimulus that activates X, say from a level of 1 to a level of 2, namely a 2-fold change. Then consider a second stimulus from 2 to 4. Both stimuli steps have the same 2-fold change, but the second step has a higher absolute change. In an FCD circuit, these two steps generate precisely the same output dynamics, including amplitude and adaptation time (Figure 4F).

FCD combines two features found in sensory systems such as vision and hearing. The first feature is Weber's law, which states

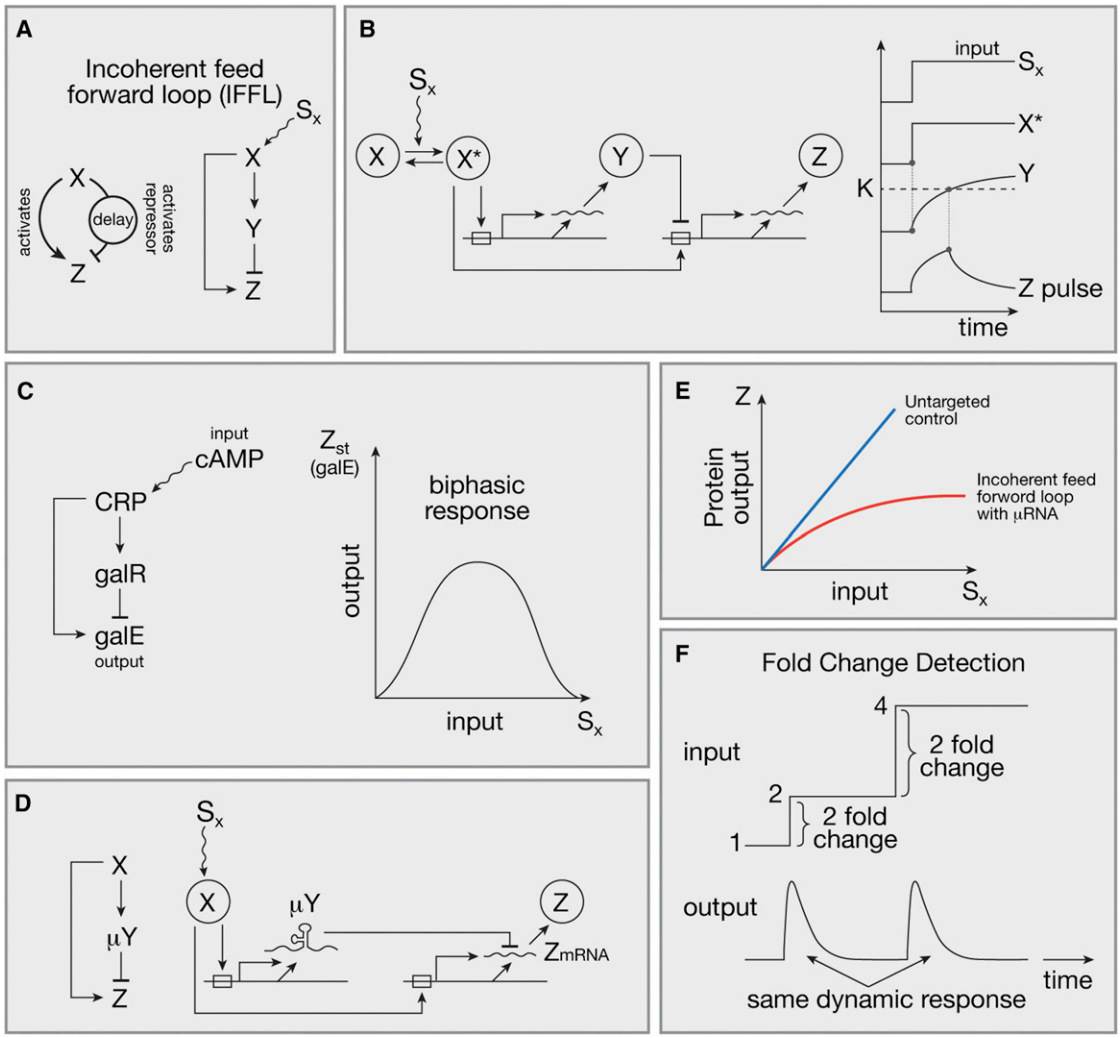

Figure 4. The IFFL Transcription Circuit Can Produce Temporal Pulses, Biphasic Responses, and FCD

(A) The IFFL in transcription regulation.

(B) An activator X activates gene Z and also activates Y, a repressor of Z. The delay due to the time it takes to make enough Y creates a pulse in the output Z.

(C) IFFLs can produce an inverse-U-shaped dose response, as exemplified in the gal system in *E. coli* (Kaplan et al., 2008).

(D) IFFLs can employ inhibitory RNAs, microRNAs, as the Y component, destabilizing Z messenger RNA and inhibiting translation.

(E) IFFLs with a microRNA inhibitor as the Y component can exhibit insensitivity to X activator levels. A synthetic circuit of IFFL with microRNA was found to make the output level insensitive to the dose of transfected vector bearing all components of the circuit (Bleris et al., 2011).

(F) IFFLs can respond to relative changes in input, rather than absolute changes, and exhibit exact adaptation in which output returns to baseline levels despite the presence of the input. This is known as FCD: a step change of input signal from level 1 to 2 produces the same output dynamics as a step increase from 2 to 4, because both steps have the same fold change (F = 2 in this example). Different fold changes produce pulses of different amplitude and duration.

that the amplitude of the response is proportional to the fold change in the signal. The second feature is exact adaptation, a response that slowly returns to a fixed baseline despite the continued presence of the input signal (Levchenko and Iglesias, 2002; Ma et al., 2009). FCD has been experimentally found in bacterial chemotaxis (Lazova et al., 2011; Masson et al., 2012; Hamadeh et al., 2013; Kojadinovic et al., 2013), wherein it is generated by a specific feedback (not feedforward) mechanism, over a range of several orders of magnitude of signal levels.

That FCD can be generated by IFFLs is, thus far, only a theoretical suggestion, yet to be tested experimentally. IFFLs are predicted to perform FCD within a certain range of parameters—for example, when Y is a strong repressor of Z. If this function exists in cells, it might help explain features of the wnt and ERK systems, which appear to respond to fold changes in signaling molecule activity (Cohen-Saidon et al., 2009; Goentoro et al., 2009). Following a step change in the input signal, FCD generated by IFFLs should have the following hallmarks: an increase in the amplitude of the response with the fold change in signal, and a (mild) decrease in the timing of the peak and the time required to adapt exactly back to steady state with the fold change in signal. These features may be found in the study of Takeda et al. (2012) on an IFFL in *Dictyostelium discoideum* chemotaxi signaling (see also Skataric and Sontag, 2012).

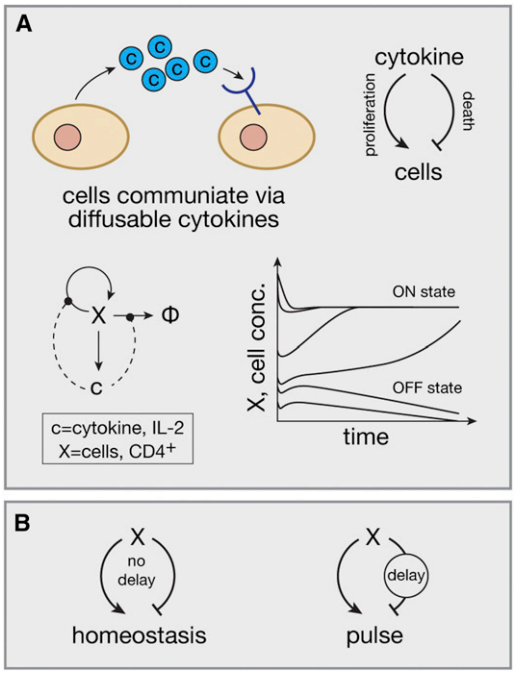

Figure 5. Cells Circuits with Paradoxical Cytokines Can Provide Homeostatic Cell Concentrations that Are Independent of Initial Cell Levels and at the Same Time Resist False-Positive Activation

(A) Immune cells (such as T helper cells) secrete a cytokine (such as IL-2) that increases both their proliferation and their death. The paradoxical nature of the cytokine can provide a homeostatic ON state in which cell steady-state levels are insensitive to initial cell levels above some cell-concentration threshold. If initial cell levels aren't high enough, the system decays to an OFF state with zero or low cell levels.

(B) A paradoxical-component design principle: When the two antagonistic activities act on the same timescale, the system can show robust homeostasis. If there is a delay in the activity of one of the antagonistic activities, a robust pulse can be generated.

Some of the features carried out by the IFFL can also be achieved by other circuit designs. For example, negative autoregulation can also speed responses (Rosenfeld et al., 2002) and reduce noise (Becskei and Serrano, 2000; Dublanche et al., 2006). FCD can be achieved by certain nonlinear feedback loops (Shoval et al., 2010). However, feedback loops are prone to become unstable if the system has delays, as often occurs in transcription circuits, leading to oscillations instead of a stable steady state (Elowitz and Leibler, 2000). In contrast, the IFFL is a feedforward circuit with no feedback and can be proved to be globally stable even in the presence of delays (Russo and Slotine, 2011). Of all feedforward circuits, the IFFL is the smallest (least number of components) that carries out its functions. Thus, it may be prevalent because of its small size and stability (Prill et al., 2005).

Antagonistically Pleiotropic Cytokines in Immune Cell Circuits

Up to now, we discussed circuits whose components are molecules. We next consider circuits on a different level of organization—circuits whose components are cells (Altan-Bonnet, 2012). Examples are provided by the immune system, in which cells communicate by means of small diffusible proteins called cytokines. Many cytokines are pleiotropic, causing two or more different effects (Janeway et al., 2004). We focus on cytokines whose effects are contradictory, such as increasing both cell growth and cell death (Cantrell and Smith, 1984; Lenardo, 1991; Li et al., 2001) (Figure 5A).

Hart et al. (2012) studied cell circuits theoretically and suggested that antagonistic pleiotropy of cytokines can provide features required by the adaptive immune response—on the one hand, rapid growth of the number of immune cells to match rapid pathogen growth; on the other hand, precision: the number of cells must not be too high in order to avoid collateral damage to the healthy tissue. Moreover, the system must resist false positives—erroneous triggering which can lead to autoimmune disease (Altan-Bonnet, 2012).

The challenge is that cells depend sensitively on the balance between proliferation and death; an imbalance leads to explosive growth or decay to zero cells. Keeping this balance requires regulatory circuits, and paradoxical cytokines can do the job.

For example, T cells secrete interleukin-2 (IL-2), a paradoxical cytokine which enhances both their proliferation rate and their death rate (Cantrell and Smith, 1984; Lenardo, 1991; Li et al., 2001). Hart et al. (2012) showed theoretically that this design allows T cells to reach a fixed final concentration at which IL-2-linked growth and death cancel out. This cell concentration is stable because a fluctuation that adds cells leads to more death; likewise, a fluctuation that removes cells leads to more proliferation, converging again on the steady-state concentration (Figure 5A).

This stable "ON" state is useful when a large T cell response is warranted. It could, however, be a liability if the ON state is reached by mistake due to a tiny initial fluctuation. The paradoxical nature of IL-2 ensures the avoidance of such false activation: starting the circuit with very few cells leads to a zero final steady state with no cells, the "OFF" state. The system is thus bistable, showing growth to a stable ON state when initial cell levels are high enough, and otherwise a decay to a zero-cell OFF state (Figure 5A). This bistabilty is due to the bifunctional nature of IL-2, which creates both a negative and a positive feedback loop on the T cells.

A second example analyzed by Hart et al. addresses the differentiation of cells from a population of precursors. Generally, the number of differentiated cells is proportional to the number of precursors. Paradoxical components can help to provide robustness of final cell numbers to variations in precursor levels. In the T cell differentiation process, the paradoxical component is the precursor cell itself. It both gives rise to the differentiated T cell and inhibits the same differentiation process by uptake of a prodifferentiation cytokine (Di Fiore and De Camilli, 2001; Sakaguchi, 2004; Pandiyan et al., 2007; Busse et al., 2010). As a result, the final level of differentiated cells becomes independent of (robust to) the concentration of precursors. A change in the number of precursors has two contradictory effects that cancel out: the change in cytokine level cancels out the change in differentiation rate.

Finally, cell circuits can form IFFLs, as in the T helper 17 cell differentiation system, potentially functioning as pulse generators or fold-change detectors (Basu et al., 2004; Li and Flavell, 2008; McGeachy and Cua, 2008; Shoval et al., 2010).

Paradoxical components on the cell or tissue level may also occur outside of immunology. For example, glucose has a paradoxical effect on beta cells in the pancreas, promoting both their proliferation and death (Dadon et al., 2012). Given that the pancreas acts to control glucose levels, this opens the possibility for an interesting circuit design. It is probable that many more principles of cell circuits await discovery.

Paradoxical Components with a Delay Cause Pulses; Those with No Delay Cause Robustness

A unifying principle emerges from these studies: When the paradoxical component carries out its two antagonistic functions at a delay, it generates a pulse of output. When there is no delay, the component can generate robustness or homeostasis (Figure 5B).

Discussion

Paradoxical components can provide biological circuits with useful functions. Bifunctional enzymes that carry out opposing reactions provide signaling and metabolic circuits with robust input-output relationships: the output depends on input signal, but not on the concentrations of any of the proteins in the circuit. This allows accurate control in the face of naturally occurring variations in the concentrations of proteins and metabolites. Bifunctional morphogen inhibitors, which also increase morphogen range, can lead to robust patterns and scaling in development. On the level of circuits made of cells, paradoxical cytokines that signal immune cells to both proliferate and die can provide robust cell concentrations. When the two antagonistic effects of a bifunctional component act on two different timescales, other features can arise: a paradoxical transcription factor in incoherent feedforward loops can provide pulses of output, nonmonotonic input-output relationships, and detection of relative rather than absolute signal changes.

Many of these functions have been experimentally demonstrated in several systems; much more testing remains to be done. This review has aimed to highlight potential areas of future interest. It would be instructive to see experiments that replace a bifunctional component with two distinct components, each carrying out only one of the functions. Robustness features are predicted in this case to be reduced or lost. Some paradoxical components are widespread, such as incoherent feedforward loops. Others are known only in a few systems, such as antagonistically bifunctional enzymes. It would be interesting to devise new ways to detect bifunctional enzymes or bifunctional enzyme complexes (Figure 2D). Finally, study aimed at understanding circuits made of cells that communicate by paradoxical cytokines and other signals is a promising direction that may lead to new principles of computation on the intercellular level.

ACKNOWLEDGMENTS

U.A. is the incumbent of the Abisch-Frenkel Professorial Chair. U.A. thanks the European Research Council (FP7) and the Israel Science Foundation for their support.

REFERENCES

Alon, U. (2007). Network motifs: theory and experimental approaches. Nat. Rev. Genet. *8*, 450–461.

Altan-Bonnet, G. (2012). Systems immunology: a primer for biophysicists. In Comprehensive Biophysics, Volume 9: Simulation and Modeling, Edward H. Egelman, ed. (Amsterdam: Elsevier), pp. 389–413.

Basu, S., Mehreja, R., Thiberge, S., Chen, M.-T., and Weiss, R. (2004). Spatiotemporal control of gene expression with pulse-generating networks. Proc. Natl. Acad. Sci. USA *101*, 6355–6360.

Batchelor, E., and Goulian, M. (2003). Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system. Proc. Natl. Acad. Sci. USA *100*, 691–696.

Becskei, A., and Serrano, L. (2000). Engineering stability in gene networks by autoregulation. Nature *405*, 590–593.

Ben-Zvi, D., and Barkai, N. (2010). Scaling of morphogen gradients by an expansion-repression integral feedback control. Proc. Natl. Acad. Sci. USA *107*, 6924–6929.

Ben-Zvi, D., Shilo, B.-Z., Fainsod, A., and Barkai, N. (2008). Scaling of the BMP activation gradient in Xenopus embryos. Nature *453*, 1205–1211.

Ben-Zvi, D., Shilo, B.-Z., and Barkai, N. (2011). Scaling of morphogen gradients. Curr. Opin. Genet. Dev. *21*, 704–710.

Blake, W.J., KAErn, M., Cantor, C.R., and Collins, J.J. (2003). Noise in eukaryotic gene expression. Nature *422*, 633–637.

Blake, W.J., Balázsi, G., Kohanski, M.A., Isaacs, F.J., Murphy, K.F., Kuang, Y., Cantor, C.R., Walt, D.R., and Collins, J.J. (2006). Phenotypic consequences of promoter-mediated transcriptional noise. Mol. Cell *24*, 853–865.

Bleris, L., Xie, Z., Glass, D., Adadey, A., Sontag, E., and Benenson, Y. (2011). Synthetic incoherent feedforward circuits show adaptation to the amount of their genetic template. Mol. Syst. Biol. *7*, 519.

Boyer, L.A., Lee, T.I., Cole, M.F., Johnstone, S.E., Levine, S.S., Zucker, J.P., Guenther, M.G., Kumar, R.M., Murray, H.L., Jenner, R.G., et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. Cell *122*, 947–956.

Brauer, M.J., Yuan, J., Bennett, B.D., Lu, W., Kimball, E., Botstein, D., and Rabinowitz, J.D. (2006). Conservation of the metabolomic response to starvation across two divergent microbes. Proc. Natl. Acad. Sci. USA *103*, 19302–19307.

Busse, D., de la Rosa, M., Hobiger, K., Thurley, K., Flossdorf, M., Scheffold, A., and Höfer, T. (2010). Competing feedback loops shape IL-2 signaling between helper and regulatory T lymphocytes in cellular microenvironments. Proc. Natl. Acad. Sci. USA *107*, 3058–3063.

Cantrell, D.A., and Smith, K.A. (1984). The interleukin-2 T-cell system: a new cell growth model. Science *224*, 1312–1316.

Capra, E.J., and Laub, M.T. (2012). Evolution of two-component signal transduction systems. Annu. Rev. Microbiol. *66*, 325–347.

Chastain, C.J., and Chollet, R. (2003). Regulation of pyruvate, orthophosphate dikinase by ADP-/Pi-dependent reversible phosphorylation in C_3 and C_4 plants. Plant Physiol. Biochem. *41*, 523–532.

Chevrier, N., Mertins, P., Artyomov, M.N., Shalek, A.K., Iannacone, M., Ciaccio, M.F., Gat-Viks, I., Tonti, E., DeGrace, M.M., Clauser, K.R., et al. (2011). Systematic discovery of TLR signaling components delineates viral-sensing circuits. Cell *147*, 853–867.

Cohen-Saidon, C., Cohen, A.A., Sigal, A., Liron, Y., and Alon, U. (2009). Dynamics and variability of ERK2 response to EGF in individual living cells. Mol. Cell *36*, 885–893.

Dadon, D., Tornovsky-Babaey, S., Furth-Lavi, J., Ben-Zvi, D., Ziv, O., Schyr-Ben-Haroush, R., Stolovich-Rain, M., Hija, A., Porat, S., Granot, Z., et al. (2012). Glucose metabolism: key endogenous regulator of β-cell replication and survival. Diabetes Obes. Metab. *14*(*Suppl 3*), 101–108.

Davidson, E.H. (2010). Emerging properties of animal gene regulatory networks. Nature *468*, 911–920.

Dennis, P.P., Ehrenberg, M., and Bremer, H. (2004). Control of rRNA synthesis in Escherichia coli: a systems biology approach. Microbiol. Mol. Biol. Rev. *68*, 639–668.

Dexter, J.P., and Gunawardena, J. (2012). Dimerization and bifunctionality confer robustness to the isocitrate dehydrogenase regulatory system in Escherichia coli. J. Biol. Chem. Published online November 28, 2012. http://dx.doi.org/10.1074/jbc.M112.339226.

Di Fiore, P.P., and De Camilli, P. (2001). Endocytosis and signaling. an inseparable partnership. Cell *106*, 1–4.

Dublanche, Y., Michalodimitrakis, K., Kümmerer, N., Foglierini, M., and Serrano, L. (2006). Noise in transcription negative feedback loops: simulation and experimental analysis. Mol. Syst. Biol. *2*, 41.

Ebert, M.S., and Sharp, P.A. (2012). Roles for microRNAs in conferring robustness to biological processes. Cell *149*, 515–524.

Eichenberger, P., Fujita, M., Jensen, S.T., Conlon, E.M., Rudner, D.Z., Wang, S.T., Ferguson, C., Haga, K., Sato, T., Liu, J.S., and Losick, R. (2004). The program of gene transcription for a single differentiating cell type during sporulation in Bacillus subtilis. PLoS Biol. *2*, e328.

Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B.-Z., and Barkai, N. (2002). Robustness of the BMP morphogen gradient in Drosophila embryonic patterning. Nature *419*, 304–308.

Elowitz, M.B., and Leibler, S. (2000). A synthetic oscillatory network of transcriptional regulators. Nature *403*, 335–338.

Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic gene expression in a single cell. Science *297*, 1183–1186.

Entus, R., Aufderheide, B., and Sauro, H.M. (2007). Design and implementation of three incoherent feed-forward motif based biological concentration sensors. Syst. Synth. Biol. *1*, 119–128.

Gerstein, M.B., Kundaje, A., Hariharan, M., Landt, S.G., Yan, K.-K., Cheng, C., Mu, X.J., Khurana, E., Rozowsky, J., Alexander, R., et al. (2012). Architecture of the human regulatory network derived from ENCODE data. Nature *489*, 91–100.

Ghosh, B., Karmakar, R., and Bose, I. (2005). Noise characteristics of feed forward loops. Phys. Biol. *2*, 36–45.

Goentoro, L., Shoval, O., Kirschner, M.W., and Alon, U. (2009). The incoherent feedforward loop can provide fold-change detection in gene regulation. Mol. Cell *36*, 894–899.

Goldberg, S.D., Clinthorne, G.D., Goulian, M., and DeGrado, W.F. (2010). Transmembrane polar interactions are required for signaling in the Escherichia coli sensor kinase PhoQ. Proc. Natl. Acad. Sci. USA *107*, 8141–8146.

Hamadeh, A., Ingalls, B., and Sontag, E. (2013). Transient dynamic phenotypes as criteria for model discrimination: fold-change detection in Rhodobacter sphaeroides chemotaxis. J. R. Soc. Interface *10*, 20120935.

Hart, Y., Madar, D., Yuan, J., Bren, A., Mayo, A.E., Rabinowitz, J.D., and Alon, U. (2011a). Robust control of nitrogen assimilation by a bifunctional enzyme in E. coli. Mol. Cell *41*, 117–127.

Hart, Y., Mayo, A.E., Milo, R., and Alon, U. (2011b). Robust control of PEP formation rate in the carbon fixation pathway of C(4) plants by a bi-functional enzyme. BMC Syst. Biol. *5*, 171.

Hart, Y., Antebi, Y.E., Mayo, A.E., Friedman, N., and Alon, U. (2012). Design principles of cell circuits with paradoxical components. Proc. Natl. Acad. Sci. USA *109*, 8346–8351.

Haskel-Ittah, M., Ben-Zvi, D., Branski-Arieli, M., Schejter, E.D., Shilo, B.-Z., and Barkai, N. (2012). Self-organized shuttling: generating sharp dorsoventral polarity in the early Drosophila embryo. Cell *150*, 1016–1028.

Hengge, R. (2009). Principles of c-di-GMP signalling in bacteria. Nat. Rev. Microbiol. *7*, 263–273.

Hornstein, E., and Shomron, N. (2006). Canalization of development by microRNAs. Nat. Genet. Suppl. *38*, S20–S24.

Hsing, W., Russo, F.D., Bernd, K.K., and Silhavy, T.J. (1998). Mutations that alter the kinase and phosphatase activities of the two-component sensor EnvZ. J. Bacteriol. *180*, 4538–4546.

Hunter, C.A. (2005). New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat. Rev. Immunol. *5*, 521–531.

Janeway, C., Travers, P., Walport, M., and Shlomchik, M. (2004). Immunobiology, Sixth Edition (New York: Garland Science).

Johnston, R.J., Jr., Otake, Y., Sood, P., Vogt, N., Behnia, R., Vasiliauskas, D., McDonald, E., Xie, B., Koenig, S., Wolf, R., et al. (2011). Interlocked feedforward loops control cell-type-specific Rhodopsin expression in the Drosophila eye. Cell *145*, 956–968.

Kaplan, S., Bren, A., Dekel, E., and Alon, U. (2008). The incoherent feed-forward loop can generate non-monotonic input functions for genes. Mol. Syst. Biol. *4*, 203.

Karp, R.L., Pérez Millán, M., Dasgupta, T., Dickenstein, A., and Gunawardena, J. (2012). Complex-linear invariants of biochemical networks. J. Theor. Biol. *311*, 130–138.

Kashtan, N., Itzkovitz, S., Milo, R., and Alon, U. (2004). Topological generalizations of network motifs. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *70*, 031909.

Kim, D., Kwon, Y.-K., and Cho, K.-H. (2008). The biphasic behavior of incoherent feed-forward loops in biomolecular regulatory networks. Bioessays *30*, 1204–1211.

Kittisopikul, M., and Süel, G.M. (2010). Biological role of noise encoded in a genetic network motif. Proc. Natl. Acad. Sci. U.S.A. *107*, 13300–13305.

Kojadinovic, M., Armitage, J.P., Tindall, M.J., and Wadhams, G.H. (2013). Rhodobacter sphaeroides chemotaxis response kinetics: complexities in signalling but similarities in responses. J. R. Soc. Interface *10*.

Krejcí, A., Bernard, F., Housden, B.E., Collins, S., and Bray, S.J. (2009). Direct response to Notch activation: signaling crosstalk and incoherent logic. Sci. Signal. *2*, ra1.

LaPorte, D.C., Thorsness, P.E., and Koshland, D.E., Jr. (1985). Compensatory phosphorylation of isocitrate dehydrogenase. A mechanism for adaptation to the intracellular environment. J. Biol. Chem. *260*, 10563–10568.

Lazova, M.D., Ahmed, T., Bellomo, D., Stocker, R., and Shimizu, T.S. (2011). Response rescaling in bacterial chemotaxis. Proc. Natl. Acad. Sci. USA *108*, 13870–13875.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., et al. (2002). Transcriptional regulatory networks in Saccharomyces cerevisiae. Science *298*, 799–804.

Lenardo, M.J. (1991). Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. Nature *353*, 858–861.

Levchenko, A., and Iglesias, P.A. (2002). Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils. Biophys. J. *82*, 50–63.

Li, M.O., and Flavell, R.A. (2008). Contextual regulation of inflammation: a duet by transforming growth factor-β and interleukin-10. Immunity *28*, 468–476.

Li, X., Cassidy, J.J., Reinke, C.A., Fischboeck, S., and Carthew, R.W. (2009). A microRNA imparts robustness against environmental fluctuation during development. Cell *137*, 273–282.

Li, X.C., Demirci, G., Ferrari-Lacraz, S., Groves, C., Coyle, A., Malek, T.R., and Strom, T.B. (2001). IL-15 and IL-2: a matter of life and death for T cells in vivo. Nat. Med. *7*, 114–118.

Ma, W., Trusina, A., El-Samad, H., Lim, W.A., and Tang, C. (2009). Defining network topologies that can achieve biochemical adaptation. Cell *138*, 760–773.

Macía, J., Widder, S., and Solé, R. (2009). Specialized or flexible feed-forward loop motifs: a question of topology. BMC Syst. Biol. *3*, 84.

Mangan, S., and Alon, U. (2003). Structure and function of the feed-forward loop network motif. Proc. Natl. Acad. Sci. USA *100*, 11980–11985.

Mangan, S., Itzkovitz, S., Zaslaver, A., and Alon, U. (2006). The incoherent feed-forward loop accelerates the response-time of the gal system of Escherichia coli. J. Mol. Biol. *356*, 1073–1081.

Masson, J.-B., Voisinne, G., Wong-Ng, J., Celani, A., and Vergassola, M. (2012). Noninvasive inference of the molecular chemotactic response using bacterial trajectories. Proc. Natl. Acad. Sci. U.S.A. *109*, 1802–1807.

McAdams, H.H., and Shapiro, L. (1995). Circuit simulation of genetic networks. Science *269*, 650–656.

McGeachy, M.J., and Cua, D.J. (2008). Th17 cell differentiation: the long and winding road. Immunity *28*, 445–453.

Miller, A.L., and Bement, W.M. (2009). Regulation of cytokinesis by Rho GTPase flux. Nat. Cell Biol. *11*, 71–77.

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D., and Alon, U. (2002). Network motifs: simple building blocks of complex networks. Science *298*, 824–827.

Mishima, M., Kaitna, S., and Glotzer, M. (2002). Central spindle assembly and cytokinesis require a kinesin-like protein/RhoGAP complex with microtubule bundling activity. Dev. Cell *2*, 41–54.

Newman, J.R.S., Ghaemmaghami, S., Ihmels, J., Breslow, D.K., Noble, M., DeRisi, J.L., and Weissman, J.S. (2006). Single-cell proteomic analysis of S. cerevisiae reveals the architecture of biological noise. Nature *441*, 840–846.

Osella, M., Bosia, C., Corá, D., and Caselle, M. (2011). The role of incoherent microRNA-mediated feedforward loops in noise buffering. PLoS Comput. Biol. *7*, e1001101.

Pandiyan, P., Zheng, L., Ishihara, S., Reed, J., and Lenardo, M.J. (2007). CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat. Immunol. *8*, 1353–1362.

Paulsson, J. (2004). Summing up the noise in gene networks. Nature *427*, 415–418.

Pilkis, S.J., Chrisman, T., Burgress, B., McGrane, M., Colosia, A., Pilkis, J., Claus, T.H., and el-Maghrabi, M.R. (1983). Rat hepatic 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase: a unique bifunctional enzyme. Adv. Enzyme Regul. *21*, 147–173.

Prill, R.J., Iglesias, P.A., and Levchenko, A. (2005). Dynamic properties of network motifs contribute to biological network organization. PLoS Biol. *3*, e343.

Rao, C.V., and Arkin, A.P. (2001). Control motifs for intracellular regulatory networks. Annu. Rev. Biomed. Eng. *3*, 391–419.

Raser, J.M., and O'Shea, E.K. (2005). Noise in gene expression: origins, consequences, and control. Science *309*, 2010–2013.

Rosenfeld, N., Elowitz, M.B., and Alon, U. (2002). Negative autoregulation speeds the response times of transcription networks. J. Mol. Biol. *323*, 785–793.

Russo, F.D., and Silhavy, T.J. (1993). The essential tension: opposed reactions in bacterial two-component regulatory systems. Trends Microbiol. *1*, 306–310.

Russo, G., and Slotine, J.-J.E. (2011). Symmetries, stability, and control in nonlinear systems and networks. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. *84*, 041929.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu. Rev. Immunol. *22*, 531–562.

Senior, P.J. (1975). Regulation of nitrogen metabolism in Escherichia coli and Klebsiella aerogenes: studies with the continuous-culture technique. J. Bacteriol. *123*, 407–418.

Shen-Orr, S.S., Milo, R., Mangan, S., and Alon, U. (2002). Network motifs in the transcriptional regulation network of Escherichia coli. Nat. Genet. *31*, 64–68.

Shinar, G., and Feinberg, M. (2010). Structural sources of robustness in biochemical reaction networks. Science *327*, 1389–1391.

Shinar, G., Milo, R., Martínez, M.R., and Alon, U. (2007). Input output robustness in simple bacterial signaling systems. Proc. Natl. Acad. Sci. USA *104*, 19931–19935.

Shinar, G., Rabinowitz, J.D., and Alon, U. (2009). Robustness in glyoxylate bypass regulation. PLoS Comput. Biol. *5*, e1000297.

Shoval, O., and Alon, U. (2010). SnapShot: network motifs. Cell *143*, 326–326.e1.

Shoval, O., Goentoro, L., Hart, Y., Mayo, A., Sontag, E., and Alon, U. (2010). Fold-change detection and scalar symmetry of sensory input fields. Proc. Natl. Acad. Sci. USA *107*, 15995–16000.

Sigal, A., Milo, R., Cohen, A., Geva-Zatorsky, N., Klein, Y., Liron, Y., Rosenfeld, N., Danon, T., Perzov, N., and Alon, U. (2006). Variability and memory of protein levels in human cells. Nature *444*, 643–646.

Skataric, M., and Sontag, E.D. (2012). A characterization of scale invariant responses in enzymatic networks. PLoS Comput. Biol. *8*, e1002748.

Slack, J.M.W. (1991). From Egg to Embryo: Regional Specification in Early Development, Second Edition (Cambridge: Cambridge University Press).

Somers, W.G., and Saint, R. (2003). A RhoGEF and Rho family GTPase-activating protein complex links the contractile ring to cortical microtubules at the onset of cytokinesis. Dev. Cell *4*, 29–39.

Sprinzak, D., Lakhanpal, A., Lebon, L., Santat, L.A., Fontes, M.E., Anderson, G.A., Garcia-Ojalvo, J., and Elowitz, M.B. (2010). Cis-interactions between Notch and Delta generate mutually exclusive signalling states. Nature *465*, 86–90.

Sprinzak, D., Lakhanpal, A., LeBon, L., Garcia-Ojalvo, J., and Elowitz, M.B. (2011). Mutual inactivation of Notch receptors and ligands facilitates developmental patterning. PLoS Comput. Biol. *7*, e1002069.

Stadtman, E.R., and Chock, P.B. (1977). Superiority of interconvertible enzyme cascades in metabolic regulation: analysis of monocyclic systems. Proc. Natl. Acad. Sci. USA *74*, 2761–2765.

Swiers, G., Patient, R., and Loose, M. (2006). Genetic regulatory networks programming hematopoietic stem cells and erythroid lineage specification. Dev. Biol. *294*, 525–540.

Takeda, K., Shao, D., Adler, M., Charest, P.G., Loomis, W.F., Levine, H., Groisman, A., Rappel, W.-J., and Firtel, R.A. (2012). Incoherent feedforward control governs adaptation of activated ras in a eukaryotic chemotaxis pathway. Sci. Signal. *5*, ra2.

Tyson, J.J., Chen, K.C., and Novak, B. (2003). Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. Curr. Opin. Cell Biol. *15*, 221–231.

Wang, S.T., Setlow, B., Conlon, E.M., Lyon, J.L., Imamura, D., Sato, T., Setlow, P., Losick, R., and Eichenberger, P. (2006). The forespore line of gene expression in Bacillus subtilis. J. Mol. Biol. *358*, 16–37.

Xu, Y., and Gunawardena, J. (2012). Realistic enzymology for post-translational modification: Zero-order ultrasensitivity revisited. J. Theor. Biol. *311*, 139–152.

Yüce, O., Piekny, A., and Glotzer, M. (2005). An ECT2-centralspindlin complex regulates the localization and function of RhoA. J. Cell Biol. *170*, 571–582.